Gabel
383899

```
=> fil medl,caplus,biosis,embase,wpids,ntis,compendex,inspec

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                                                         ENTRY    SESSION
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=> s (classif? or count?)(3a)leu!ocyte?

L1       40540 FILE MEDLINE
L2        3019 FILE CAPLUS
L3        6054 FILE BIOSIS
L4       12296 FILE EMBASE
L5         178 FILE WPIDS
L6          83 FILE NTIS
L7          61 FILE COMPENDEX
L8          50 FILE INSPEC

TOTAL FOR ALL FILES
L9       62281 (CLASSIF? OR COUNT?)(3A) LEU!OCYTE?

=> s ((leukocyte count or leukocyte number or lymphocyte count or
leukocytosis? or white(2a)blood cell count or blood cell count, white)/ct or
(leu!ocyte or lymphocyte)(w)(count or number))

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'WHITE(2A)BLOOD'
L10      50266 FILE MEDLINE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'WHITE(2A)BLOOD'
L11       3326 FILE CAPLUS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'WHITE(2A)BLOOD'
L12      10259 FILE BIOSIS
```

```
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'WHITE(2A)BLOOD'
L13      22646 FILE EMBASE
L14        125 FILE WPIDS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'WHITE(2A)BLOOD'
L15        114 FILE NTIS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'WHITE(2A)BLOOD'
L16         53 FILE COMPENDEX
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'WHITE(2A)BLOOD'
L17         56 FILE INSPEC

TOTAL FOR ALL FILES
L18      86845 ((LEUKOCYTE COUNT OR LEUKOCYTE NUMBER OR LYMPHOCYTE COUNT OR
               LEUKOCYTOSIS? OR WHITE(2A) BLOOD CELL COUNT OR BLOOD CELL
COUNT,
                WHITE)/CT OR (LEU!OCYTE OR LYMPHOCYTE)(W)(COUNT OR NUMBER))

=> s (l9 or l18)(l)(fluorescen? label? antibod? or anti(2w)(cd11b or cd16 or
cd66b or cd66c))

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L10)(L)(FLUORESCE'
L19         19 FILE MEDLINE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L11)(L)(FLUORESCE'
L20          1 FILE CAPLUS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L12)(L)(FLUORESCE'
L21          4 FILE BIOSIS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L13)(L)(FLUORESCE'
L22         12 FILE EMBASE
L23          0 FILE WPIDS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L15)(L)(FLUORESCE'
L24          0 FILE NTIS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L16)(L)(FLUORESCE'
L25          0 FILE COMPENDEX
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L17)(L)(FLUORESCE'
L26          0 FILE INSPEC

TOTAL FOR ALL FILES
L27         36 (L9 OR L18)(L)(FLUORESCEN? LABEL? ANTIBOD? OR ANTI(2W)(CD11B
OR
               CD16 OR CD66B OR CD66C))

=> s l27 and (fitc or fluorescein? isothiocyan? or phycoerythrin? or pe or
allophicocyanin? or apc or texas red or pe cy5 or peridinin? chlorophyll
protein or percp)

L28          2 FILE MEDLINE
L29          0 FILE CAPLUS
L30          0 FILE BIOSIS
L31          1 FILE EMBASE
L32          0 FILE WPIDS
L33          0 FILE NTIS
```

L34 0 FILE COMPENDEX
L35 0 FILE INSPEC

TOTAL FOR ALL FILES
L36 3 L27 AND (FITC OR FLUORESCEIN? ISOTHIOCYAN? OR PHYCOERYTHRIN? OR
PE OR ALLOPHICOCYANIN? OR APC OR TEXAS RED OR PE CY5 OR PERIDINI
N? CHLOROPHYLL PROTEIN OR PERCP)

=> dup rem l36

PROCESSING COMPLETED FOR L36
L37 2 DUP REM L36 (1 DUPLICATE REMOVED)

=> d 1-2 cbib abs hit

L37 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
97294030 Document Number: 97294030. Toward a new reference method for the leukocyte five-part differential. Hubl W; Wolfbauer G; Andert S; Thum G; Streicher J; Hubner C; Lapin A; Bayer P M. (Central Laboratory, Wilhelminenspital, Vienna, Austria.) CYTOMETRY, (1997 Apr 15) 30 (2) 72-84. Journal code: D92. ISSN: 0196-4763. Pub. country: United States. Language: English.

AB A flow cytometric method performing a five-part leukocyte differential based on three-color staining with anti-CD45-**fluorescein isothiocyanate** (**FITC**), anti-CD-14-**phycoerythrin** (**PE**)/**Cy5**, and a cocktail of **PE**-labeled **anti**-CD2, **anti-CD16**, and anti-HLA-DR antibodies was evaluated. Results obtained by using three different sample preparation procedures and two different flow cytometers were compared with those of a 1,000-cell manual differential for evaluation of accuracy. We observed excellent correlations with the manual differential for all leukocyte subclasses and even higher correlations between the different flow cytometric methods. Flow cytometric basophil results were identical to the manual counts, regardless of which sample preparation technique or flow cytometer was used. Therefore, we propose our flow cytometric method as the first acceptable automated reference method for basophil counting. The flow cytometric results for the other leukocyte subclasses were apparently influenced by the sample preparation, which could not be explained by cell loss during washing steps. Moreover, a small influence of the flow cytometer was also observed. Assessing the influence of sample storage, we found only minimal changes within 24 h. In establishing reference values, high precision of flow cytometric results facilitated detection of a significantly higher monocyte count for males (relative count: 7.08 +/- 1.73% vs. 6.44 +/- 1.33%, $P < 0.05$; absolute count: 0.536 +/- 0.181 x 10(9)/liter vs. 0.456 +/- 139 x 10(9)/liter, $P < 0.01$). Our data indicate that monoclonal antibody-based flow cytometry is a highly suitable reference method for the five-part differential: It also shows, however, that studies will have to put more emphasis on methodological issues to define a method that shows a high interlaboratory reproducibility.

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CT Check Tags: Comparative Study; Human
Antibodies, Monoclonal
Antigens, CD: AN, analysis
*Flow Cytometry: MT, methods
Fluorescent Antibody Technique, Direct
***Leukocyte Count: MT, methods**
Leukocytes: CY, cytology
Leukocytes: IM, immunology
Reference Values
Regression Analysis
Reproducibility of Results

L37 ANSWER 2 OF 2 MEDLINE

91035905 Document Number: 91035905. A study of granular lymphocytes (GL) in children and young adults. Takehana J; Fukunaga Y. (Department of Pediatrics, Nippon Medical School..) NIPPON IKA DAIGAKU ZASSHI. JOURNAL OF THE NIPPON MEDICAL SCHOOL, (1990 Aug) 57 (4) 322-33. Journal code: HRD. ISSN: 0048-0444. Pub. country: Japan. Language: Japanese.

AB In this study, we investigate the percentage and absolute number of granular lymphocytes (GL) in venous blood smears and the percentage of CD16+ and CD57+ lymphocytes in the peripheral venous blood of 91 healthy controls (1 to 29 years old) and 168 patients with various diseases (1 month to 36 years old). GL were morphologically classified into four subsets: large-sized lymphocytes with large granules (LL-LG), large-sized lymphocytes with small granules (LL-SG), medium-sized lymphocytes with large granules (ML-LG) and medium-sized lymphocytes with small granules (ML-SG). The results were as follows: 1) In the controls, the percentage of GL in total lymphocytes was 10.0 +/- 6.2% (mean +/- 1SD). Therefore its normal value was below 22.4% (mean +/- 2SD). The absolute count of GL was 320.7 +/- 221.0/mm3. The percentages of LL-LG, LL-SG, ML-LG and ML-SG in total lymphocytes were 1.7 +/- 2.2%, 2.3 +/- 2.8%, 2.6 +/- 2.3% and 3.4 +/- 2.7%, respectively. 2) In flow cytometric studies, the percentage of CD16+ in the controls was 10.2 +/- 6.7%; CD57+, 8.2 +/- 5.1%; CD57+ CD16+,

4.2 +/- 3.3%; CD57+ CD16-, 4.0 +/- 2.8%; CD57- CD16+, 6.4 +/- 4.8%. The distribution patterns of lymphocytes by two-color analysis with **FITC**-labelled **anti-CD16** and **PE**-labelled anti-CD57 monoclonal antibodies in the controls were classified into four groups. 3) Only seven of the 168 patients exhibited significantly high percentage of GL in total lymphocytes. They consisted of 4 splenectomized patients and 3 patients with pancytopenia, two of whom had pancytopenia complicated by immunoglobulin deficiency. Five of the 7 patients also had markedly high percentage of CD16+ and/or CD57+ lymphocytes. In these 5 patients, the ratios of four subsets of GL and the distribution patterns of lymphocytes with CD16 and CD57 surface antigens were different from the patient to patient and those for controls.

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CT Check Tags: Case Report; Female; Human; Male
Adolescence
Adult
Age Factors
Antigens, CD: AN, analysis
Antigens, CD: IM, immunology
Child
Child, Preschool
English Abstract
Flow Cytometry
Infant
*Killer Cells, Natural: CY, cytology
Leukocyte Count
Pancytopenia: BL, blood
Sex Factors
Splenectomy

=> s (l9 or l18) and (neutrophil? or eosinophil? or basophil? or band cell? or granulocyte?)

L38 12984 FILE MEDLINE
L39 1097 FILE CAPLUS
L40 2479 FILE BIOSIS
L41 5347 FILE EMBASE
L42 54 FILE WPIDS
L43 29 FILE NTIS
L44 18 FILE COMPENDEX
L45 16 FILE INSPEC

TOTAL FOR ALL FILES
L46 22024 (L9 OR L18) AND (NEUTROPHIL? OR EOSINOPHIL? OR BASOPHIL? OR BAND CELL? OR GRANULOCYTE?)

=> s l46 and (immature granulocyte? or metamyelocyte? or myelocyte? or promyelocyte?)

L47 151 FILE MEDLINE
L48 19 FILE CAPLUS
L49 42 FILE BIOSIS
L50 103 FILE EMBASE
L51 7 FILE WPIDS
L52 0 FILE NTIS
L53 0 FILE COMPENDEX
L54 0 FILE INSPEC

TOTAL FOR ALL FILES
L55 322 L46 AND (IMMATURE GRANULOCYTE? OR METAMYELOCYTE? OR MYELOCYTE? OR PROMYELOCYTE?)

=> s l55 and (leukocyte/ct or leu!ocyte? or a11.118.637/ct or a15.145.229.637/ct or a15.382.490/ct or white blood cells/ct or white blood cells or blood cells(2a)white)

L56 142 FILE MEDLINE
L57 18 FILE CAPLUS
L58 40 FILE BIOSIS
L59 91 FILE EMBASE
L60 7 FILE WPIDS
L61 0 FILE NTIS
L62 0 FILE COMPENDEX
L63 0 FILE INSPEC

TOTAL FOR ALL FILES
L64 298 L55 AND (LEUKOCYTE/CT OR LEU!OCYTE? OR A11.118.637/CT OR A15.145
.229.637/CT OR A15.382.490/CT OR WHITE BLOOD CELLS/CT OR WHITE BLOOD CELLS OR BLOOD CELLS(2A) WHITE)

=> s l64 and (flow cytome? or flow cytometry/ct or cytomet? or e5.196.172.382.240.350/ct or e5.909.262.386.350/ct)

L65 4 FILE MEDLINE
L66 0 FILE CAPLUS
L67 2 FILE BIOSIS
L68 6 FILE EMBASE
L69 2 FILE WPIDS

L70 0 FILE NTIS
L71 0 FILE COMPENDEX
L72 0 FILE INSPEC

TOTAL FOR ALL FILES
L73 14 L64 AND (FLOW CYTOME? OR FLOW CYTOMETRY/CT OR CYTOMET? OR E5.196
.172.382.240.350/CT OR E5.909.262.386.350/CT)

=> dup rem l73

PROCESSING COMPLETED FOR L73
L74 10 DUP REM L73 (4 DUPLICATES REMOVED)

=> d cbib abs 1-10 hit

L74 ANSWER 1 OF 10 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
1999366789 EMBASE Targeted disruption of the murine fps/fes proto-oncogene reveals that Fps/Fes kinase activity is dispensable for hematopoiesis.
Senis Y.; Zirngibl R.; McVeigh J.; Haman A.; Hoang T.; Greer P.A.. P.A. Greer, Cancer Research Laboratories, Department of Pathology, Queen's University, Kingston, Ont. K7L 3N6, Canada. greerp@post.queensu.ca.
Molecular and Cellular Biology 19/11 (7436-7446) 1999.
Refs: 68.
ISSN: 0270-7306. CODEN: MCEBD4. Pub. Country: United States. Language: English. Summary Language: English.
AB The fps/fes proto-oncogene encodes a cytoplasmic protein-tyrosine kinase that is functionally implicated in the survival and terminal differentiation of myeloid progenitors and in signaling from several members of the cytokine receptor superfamily. To gain further insight into the physiological function of fps/fes, we targeted the mouse locus with a kinase-inactivating missense mutation. Mutant Fps/Fes protein was expressed at normal levels in these mice, but it lacked detectable kinase activity. Homozygous mutant animals were viable and fertile, and they showed no obvious defects. **Flow cytometry** analysis of bone marrow showed no statistically significant differences in the levels of myeloid, erythroid, or B-cell precursors. Subtle abnormalities observed in mutant mice included slightly elevated total **leukocyte counts** and splenomegaly. In bone marrow hematopoietic progenitor cell colony-forming assays, mutant mice gave slightly elevated numbers and variable sizes of CFU- **granulocyte** macrophage in response to interleukin-3 (IL-3) and **granulocyte**- macrophage colony-stimulating factor (GM-CSF). Tyrosine phosphorylation of Stat3 and Stat5A in bone marrow-derived macrophages was dramatically reduced in response to GM-CSF but not to IL-3 or IL-6. This suggests a distinct nonredundant role for Fps/Fes in signaling from the GM-CSF receptor that does not extend to the closely related IL-3 receptor. Lipopolysaccharide-induced Erk1/2 activation was also reduced in mutant macrophages. These subtle molecular phenotypes suggest a possible nonredundant role for Fps/Fes in myelopoiesis and immune responses.
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expressed at normal levels in these mice, but it lacked detectable kinase activity. Homozygous mutant animals were viable and fertile, and they showed no obvious defects. **Flow cytometry** analysis of bone marrow showed no statistically significant differences in the levels of myeloid, erythroid, or B-cell precursors. Subtle abnormalities observed in mutant mice included slightly elevated total **leukocyte counts** and splenomegaly. In bone marrow hematopoietic progenitor cell colony-forming assays, mutant mice gave slightly elevated numbers and variable sizes of CFU- **granulocyte** macrophage in response to interleukin-3 (IL-3) and **granulocyte**- macrophage colony-stimulating factor (GM-CSF). Tyrosine phosphorylation of Stat3 and Stat5A in bone marrow-derived macrophages was dramatically reduced in response to GM-CSF but not to IL-3 or IL-6. This suggests a distinct nonredundant role for Fps/Fes in signaling from the GM-CSF receptor that does not extend to the closely related IL-3 receptor. Lipopolysaccharide-induced Erk1/2 activation was also reduced in mutant macrophages. These subtle molecular phenotypes suggest a possible nonredundant role for Fps/Fes in myelopoiesis and immune responses.

CT Medical Descriptors:
*proto oncogene
*gene disruption
*gene targeting
*hematopoiesis
enzyme activity
signal transduction
promyelocyte
gene function
gene expression
protein phosphorylation
macrophage
gene activation
missense mutation
enzyme inactivation
nonhuman
male
female
mouse
controlled study
animal cell
embryo
article
priority journal
Drug Descriptors:
*protein tyrosine kinase
*interleukin 3
***granulocyte colony stimulating factor**
*stat3 protein
*stat5 protein
interleukin 3 receptor
interleukin 6
lipopolysaccharide

L74 ANSWER 2 OF 10 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
2000013061 EMBASE Isolation of **neutrophil** precursors from bone marrow for biochemical and transcriptional analysis. Cowland J.B.; Borregaard N.. J.B. Cowland, Finsen Centre, Department of Hematology, University of Copenhagen, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark. jcowland@rh.dk. Journal of Immunological Methods 232/1-2 (191-200) 1999.

Refs: 34.
ISSN: 0022-1759. CODEN: JIMMBG.
Publisher Ident.: S 0022-1759(99)00176-3. Pub. Country: Netherlands.
Language: English. Summary Language: English.

AB The **neutrophilic granulocyte** is the most numerous **leukocyte** in peripheral blood. The development from a multipotent progenitor cell to a mature **neutrophil** takes place in the bone marrow over a period of 10-14 days. In order to understand the cellular mechanisms behind this process, it is necessary to investigate cells from different stages of **neutrophil** differentiation. As no human cell line has the ability to faithfully reproduce the entire differentiation process from **promyelocyte** to segmented **neutrophil** the analysis of many maturation-dependent processes has to be done on **neutrophil** precursors from human bone marrow. For this purpose, a technique whereby **neutrophil** precursors can be isolated from the bone marrow and separated according to their maturity is required. Two different methods have been shown to be useful for isolation of immature **neutrophils**: density centrifugation on a Percoll gradient, where the increasing density of the cells with maturity forms the basis of the separation, and multidimensional **flow cytometry**, where a combination of size, granulation, and surface markers are used for the discrimination of different **neutrophil** precursors. This paper will review these two methods for separation of **neutrophil** precursors with special emphasis on Percoll density centrifugation and the use of cells isolated by this technique for the analysis of **neutrophil**-specific mRNAs and the biosynthesis of **neutrophil** granule proteins.

TI Isolation of **neutrophil** precursors from bone marrow for biochemical and transcriptional analysis.

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CT Medical Descriptors:
*cell isolation
***neutrophil**
precursor cell
bone marrow cell
cell differentiation
cell maturation

granulocyte
leukocyte differential count
genetic transcription
density gradient centrifugation
human
human tissue
human cell
review
priority journal

L74 ANSWER 3 OF 10 MEDLINE
1998346195 Document Number: 98346195. The Haematology Analyser SF-3000: performance of the automated white blood cell differential count in comparison to the Haematology Analyser NE-1500. Korninger L; Mustafa G; Schwarzinger I. (Department of Laboratory Medicine, University of Vienna, Austria.) CLINICAL AND LABORATORY HAEMATOLOGY, (1998 Apr) 20 (2) 81-6. Journal code: DKF. ISSN: 0141-9854. Pub. country: ENGLAND: United Kingdom.
Language: English.
AB The present study evaluates the performance of automated white blood cell (WBC) differential counts by the new Haematology Analyser SF-3000. Five hundred and sixty-six WBC differential counts performed by the SF-3000 were compared with WBC differential counts of the well established analyser NE-1500 and to manual reference counts. Numerical results of the WBC differential counts were correlated to each other by regression analyses. The efficiency of instrument flagging for the presence of abnormal WBC was expressed as per cent of subjects correctly classified. **Neutrophil** and **lymphocyte counts** correlated well between analysers and to manual reference counts. Monocyte counts for the SF-3000 correlated significantly better with the microscopic counts, whereas correlations of **eosinophils** and **basophils** were better for the NE-1500. The efficiency rates of flagging for the presence of > or = 1% abnormal WBC were 80% for the NE-1500 and 70% for the SF-3000. This difference was exclusively due to low specificity of the SF-3000 in flagging cells of the 'Left Shift' category, especially in samples with elevated WBC counts. The flagging efficiencies for blasts, **promyelocytes**, **myelocytes**, atypical lymphocytes and nucleated red cells were identical for both analysers. Thus, with regard to the performance of automated WBC differential counts the SF-3000 seems comparable with other, well established haematology analysers.
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to the performance of automated WBC differential counts the SF-3000 seems comparable with other, well established haematology analysers.

CT Check Tags: Comparative Study; Human
Automation
Blood Specimen Collection
False Positive Reactions
Flow Cytometry: IS, instrumentation
Hematologic Diseases: BL, blood
Hematologic Diseases: DI, diagnosis
Lasers
***Leukocyte Count: IS, instrumentation**
Leukocytes: CL, classification
Leukocytes: UL, ultrastructure
Reference Standards

L74 ANSWER 4 OF 10 MEDLINE DUPLICATE 1

97317517 Document Number: 97317517. Blood concentrations of G-CSF and myelopoiesis in patients undergoing aortocoronary bypass surgery. Usui A; Kawamura M; Hibi M; Yoshida K; Murakami F; Tomita Y; Ooshima H; Murase M. (Department of Thoracic Surgery, Nagoya University, School of Medicine, Japan.) ANNALS OF HEMATOLOGY, (1997 Apr) 74 (4) 169-73. Journal code: A2P. ISSN: 0939-5555. Pub. country: GERMANY: Germany, Federal Republic of.

Language: English.

AB The pattern of changes in **leukocyte counts** and the blood concentration of G-CSF were observed in 15 patients undergoing aortocoronary bypass surgery. Myelopoietic function was assessed by examining the myelogram and performing **flow cytometry** to identify human **leukocyte** differentiation antigens on bone marrow aspirates obtained from the sternum when opening and closing the sternotomy. The blood concentration of G-CSF increased gradually after removal of the aortic cross clamp and peaked on the first postoperative day (232 +/- 98 ngml). The white blood cell count also increased during the operation and peaked on the second postoperative day, demonstrating a threefold increase (15,800 +/- 2700). **Granulocytes** represented most of the increase, while lymphocytes and monocytes showed no significant changes. The myelogram showed that the percentages of myeloblasts, **promyelocytes**, and **metamyelocytes** did not change; however, the percentage of **myelocytes** increased significantly during surgery (14.0 +/- 2.5% vs. 17.3 +/- 3.5%, $p < 0.05$). The number of mature **myelocytes** (LFA-1 beta and Leu-15 positive) decreased significantly ($p < 0.01$ and $p < 0.05$) during surgery. With the two-color method, the ratio of immature **myelocytes** (MCS-2 negative and Leu-15 negative) increased significantly ($p < 0.01$). The ratio of myeloblasts (Leu-11 and HLA-DR positive) and the ratio of stem cells (CD 34 and MY-9 positive) did not increase significantly during the operation. G-CSF concentrations increase substantially during aortocoronary bypass surgery and may be responsible for the rise in **granulocyte** and total **leukocyte counts**, as well as for the increase in immature **myelocytes** seen on bone marrow examination.

AB The pattern of changes in **leukocyte counts** and the blood concentration of G-CSF were observed in 15 patients undergoing aortocoronary bypass surgery. Myelopoietic function was assessed by examining the myelogram and performing **flow cytometry** to identify human **leukocyte** differentiation antigens on bone marrow aspirates obtained from the sternum when opening and closing the sternotomy. The blood concentration of G-CSF increased gradually after removal of the aortic cross clamp and peaked on the first postoperative day (232 +/- 98 ngml). The white blood cell count also increased during the operation and peaked on the second postoperative day, demonstrating a

threefold increase (15,800 +/- 2700). **Granulocytes** represented most of the increase, while lymphocytes and monocytes showed no significant changes. The myelogram showed that the percentages of myeloblasts, **promyelocytes**, and **metamyelocytes** did not change; however, the percentage of **myelocytes** increased significantly during surgery (14.0 +/- 2.5% vs. 17.3 +/- 3.5%, $p < 0.05$). The number of mature **myelocytes** (LFA-1 beta and Leu-15 positive) decreased significantly ($p < 0.01$ and $p < 0.05$) during surgery. With the two-color method, the ratio of immature **myelocytes** (MCS-2 negative and Leu-15 negative) increased significantly ($p < 0.01$). The ratio of myeloblasts (Leu-11 and HLA-DR positive) and the ratio of stem cells (CD 34 and MY-9 positive) did not increase significantly during the operation. G-CSF concentrations increase substantially during aortocoronary bypass surgery and may be responsible for the rise in **granulocyte** and total **leukocyte counts**, as well as for the increase in immature **myelocytes** seen on bone marrow examination.

CT Check Tags: Female; Human; Male
Aged
Antigens, Differentiation: AN, analysis
Bone Marrow: CY, cytology
*Bone Marrow: GD, growth & development
Bone Marrow: IM, immunology
*Coronary Artery Bypass
Flow Cytometry
***Granulocyte Colony-Stimulating Factor: BL, blood**
*Hematopoiesis: DE, drug effects
Leukocyte Count
Lymphocyte Function-Associated Antigen-1: AN, analysis
Middle Age

RN **143011-72-7 (Granulocyte Colony-Stimulating Factor)**

L74 ANSWER 5 OF 10 MEDLINE DUPLICATE 2

94317621 Document Number: 94317621. Flow-cytochemical differential **leukocyte** analysis with quantitation of **neutrophil** left shift. An evaluation of the Cobas-Helios analyzer. Bentley S A; Johnson T S; Sohier C H; Bishop C A. (Department of Pathology, University of North Carolina School of Medicine, Chapel Hill 27514..) AMERICAN JOURNAL OF CLINICAL PATHOLOGY, (1994 Aug) 102 (2) 223-30. Journal code: 3FK. ISSN: 0002-9173. Pub. country: United States. Language: English.

AB The Cobas-Helios (Roche Diagnostic Systems, Inc., Branchburg, NJ) is a new, fully automated hematology analyzer that performs a complete blood **count** and differential **leukocyte count** (DLC), **classifying leukocytes** by flow-cytochemical technology. The DLC component of the Cobas-Helios was evaluated according to the National Committee for Clinical Laboratory Standards H20-A protocol. Instrument performance was acceptable with respect to all parameters investigated, including imprecision, inaccuracy and clinical sensitivity for the identification of quantitative and qualitative **leukocyte** abnormalities. In a minority of samples with **neutrophil** left shift, **neutrophils** tended to overlap the monocyte domain, resulting in overestimation of monocytes and underestimation of **neutrophils**. This problem did not affect clinical sensitivity and was generally associated with a positive instrumental left-shift flag. Flags for the identification of specific qualitative abnormalities of the **leukocyte** population (atypical lymphoid cells, nucleated red cells, blast cells, **immature granulocytes** and **neutrophil** left shift) performed well. In addition to a conventional five-part DLC, the Cobas-Helios also identifies and quantitates atypical lymphoid cells and "large immature cells," the latter

corresponding to bands and **immature granulocytes**.
Counts of atypical lymphoid cells and large immature cells correlated well
with the equivalent cell classes as enumerated by the reference method of the National Committee for Clinical Laboratory Standards. The Cobas-Helios
offers the most reliable quantitative index of **neutrophil** left shift currently available in a commercial automated DLC analyzer.

TI Flow-cytochemical differential **leukocyte** analysis with quantitation of **neutrophil** left shift. An evaluation of the Cobas-Helios analyzer.

AB The Cobas-Helios (Roche Diagnostic Systems, Inc., Branchburg, NJ) is a new, fully automated hematology analyzer that performs a complete blood **count** and differential **leukocyte count** (DLC), **classifying leukocytes** by flow-cytochemical technology. The DLC component of the Cobas-Helios was evaluated according to the National Committee for Clinical Laboratory Standards H20-A protocol. Instrument performance was acceptable with respect to all parameters investigated, including imprecision, inaccuracy and clinical sensitivity for the identification of quantitative and qualitative **leukocyte** abnormalities. In a minority of samples with **neutrophil** left shift, **neutrophils** tended to overlap the monocyte domain, resulting in overestimation of monocytes and underestimation of **neutrophils**. This problem did not affect clinical sensitivity and was generally associated with a positive instrumental left-shift flag. Flags for the identification of specific qualitative abnormalities of the **leukocyte** population (atypical lymphoid cells, nucleated red cells, blast cells, **immature granulocytes** and **neutrophil** left shift) performed well. In addition to a conventional five-part DLC, the Cobas-Helios also identifies and quantitates atypical lymphoid cells and "large immature cells," the latter
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Counts of atypical lymphoid cells and large immature cells correlated well
with the equivalent cell classes as enumerated by the reference method of the National Committee for Clinical Laboratory Standards. The Cobas-Helios
offers the most reliable quantitative index of **neutrophil** left shift currently available in a commercial automated DLC analyzer.

CT Check Tags: Human
Bias (Epidemiology)
Flow Cytometry: IS, instrumentation
Leukocyte Count: IS, instrumentation
Leukocytes: PA, pathology
Neutrophils: CY, cytology
Reference Values
Regression Analysis
Sensitivity and Specificity

L74 ANSWER 6 OF 10 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

94274685 EMBASE Document No.: 1994274685. Morphologic and quantitative alterations in hematopoietic cells associated with growth factor therapy: Review of the literature. Schmitz L.L.; Litz C.E.; Brunning R.D.. Dept of Lab Medicine and Pathology, Mayo Building, Box 198 UMHC, University of Minnesota Hospital, 420 Delaware Street SE,Minneapolis, MN 55455, United States. Hematologic Pathology 8/3 (55-73) 1994.
ISSN: 0886-0238. CODEN: HEPAEG. Pub. Country: United States. Language: English.

CT Medical Descriptors:
*bone marrow cell

*granulopoiesis
*hematopoietic cell
agranulocytosis
bone marrow biopsy
cancer chemotherapy
cell ultrastructure
eosinophilia
flow cytometry
hematological parameters
histiocyte
human
human cell
leukemia: DT, drug therapy
leukocyte count
leukocytosis
lymphocytopenia
monocytosis
neutropenia: DT, drug therapy
neutrophil
priority journal
promyelocyte
review
Drug Descriptors:
***granulocyte colony stimulating factor: EC, endogenous compound**
***granulocyte colony stimulating factor: PD, pharmacology**
***granulocyte colony stimulating factor: IT, drug interaction**
***granulocyte colony stimulating factor: DT, drug therapy**
***granulocyte colony stimulating factor: CB, drug combination**
***granulocyte macrophage colony stimulating factor: PD, pharmacology**
***granulocyte macrophage colony stimulating factor: CB, drug combination**
***granulocyte macrophage colony stimulating factor: IT, drug interaction**
***granulocyte macrophage colony stimulating factor: DT, drug therapy**
***granulocyte macrophage colony stimulating factor: EC, endogenous compound**
*hemopoietic growth factor: IT, drug interaction
*hemopoietic growth factor: CB, drug combination
*hemopoietic growth factor: PD, pharmacology
*hemopoietic growth factor: EC, endogenous compound
*hemopoietic growth factor: DT, drug therapy
antileukemic agent: DT, drug therapy
cd11b antigen: EC, endogenous compound
cell adhesion molecule: EC, endogenous compound
glycoprotein p 15095: EC, endogenous compound
intercellular adhesion molecule 1: EC, endogenous compound

L74 ANSWER 7 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1993-037830 [05] WPIDS
AB EP 525398 A UPAB: 19931119
Prepn. of a specimen for **classifying** and **counting** **leukocytes** into at least 8 gps., i.e. **immature** **granulocytes** (2 gps.), erythroblasts, **basophils**, **eosinophils**, lymphocytes, monocytes and **neutrophils**, by assaying a single specimen with a flow cyotmeter, comprises (a) eliminating influences of erythrocytes from a haematological sample without changing the **leukocytes** morphologically by (i) fragmenting erythrocytes contained in the sample by adding an aq. soln. of

a low osmotic pressure comprising a buffer for maintaining the pH of the soln. within an acidic range to the sample and thus damaging the cell membranes of erythroblasts, (ii) adding to the mixt. a soln. comprising an
osmolarity compensating agent for maintaining the morphology of **leukocytes** unchanged and a buffer for neutralising the acid in the first aq. soln. and adjusting the pH to a level suitable for staining and (b) staining **leukocytes** in the sample with at least 4 dyes specified as follows: (i) Astrazon Yellow 34 capable of staining at least **basophils** and **immature granulocytes**, (ii) Neutral Red capable of staining at least **eosinophils**, (iii) a dye (I) capable of staining at least either or both of the nuclei and cytoplasm of **leukocytes** and (iv) a fluorochrome (II) capable of staining exclusively the nuclei of damaged cells.

USE/ADVANTAGE - By eliminating the influences of erythrocytes, **leukocytes** can be accurately **classified** and cell vol. can be accurately measured. The method allows **classification** of **leukocytes** into at least 8 gps. and further allows sepn. of blasts.

1/8

Dwg.1/8

ABEQ US 5264369 A UPAB: 19940120

Blood specimens are prepared by (A) eliminating influences of erythrocytes without damaging **leukocytes** morphology by (a) fragmentising the erythrocytes in the sample by adding an aq. soln. of low osmotic press contg. a buffer to maintain the mixt. obtd. at an acidic pH and to damage the cell membranes of erythroblasts only and (b) adding to the mixt. an osmology compensating soln. to maintain the morphology of the **leukocytes**, which also contains a buffer to adjust the pH to a level suitable for staining and (B) staining the blood corpuscles in the sample with (c) Astrazon Yellow 3G able to stain differentially at least **basophils** and **immature granulocytes**, (d) Neutral Red able to stain differentially at least **eosinophils**, (e) a dye able to stain differentially either or both of nuclei and cytoplasm of **leukocytes** and (f) a fluorochrome dye staining exclusively nuclei of the damaged erythroblasts. The dye for (Bc) is e.g. Astra Violket, Cyanosine. The fluorochrome dye is e.g. propidium iodide.

USE - To classify and count blood gp. corpuscles into at least 8 gps., i.e. 2 comprising **immature granulocytes** gp. 1 and gp. 2, erythroblasts, **basophils**, **eosinophils**, lymphocytes, monocytes and **neutrophils** by assay of a single specimen.

Dwg. 1/8

ABEQ EP 525398 B UPAB: 19960719

A method for preparing a specimen for **classifying** and **counting leukocytes** into at least eight groups, namely, two each comprising **immature granulocytes**, one comprising erythroblasts, one comprising **basophils**, one comprising **eosinophils**, one comprising lymphocytes, one comprising monocytes and one comprising **neutrophils**, by assaying a single specimen with a **flow cytometer**, which comprises the following steps: (1) a step for eliminating influences of erythrocytes from a haematological sample without changing the **leukocytes** morphologically and comprises: (i) fragmenting erythrocytes contained in the haematological sample by adding a first aqueous solution of a low osmotic pressure comprising a buffer for maintaining the pH value of the solution within an acidic range to the haematological sample and thus damaging the cell membranes of erythroblasts; (ii) adding to the mixture obtained in (i) a second solution comprising an osmolarity compensating agent for maintaining the

morphology of **leukocytes** unchanged and a buffer for neutralising the acid in the first aqueous solution and adjusting to a ph level suitable for staining; and (2) a step for staining **leukocytes** contained in the haematological sample with at least the four dyes specified below; (i) Astrazon Yellow 3G capable of staining at least **basophils** and **immature granulocytes**; (ii) Neutral Red capable of staining at least **eosinophils**; (iii) a dye capable of staining at least either or both of the nuclei and cytoplasm of **leukocytes**; and (iv) a fluorochrome capable of staining exclusively the nuclei of damaged cells.

Dwg.1/8

TI **Classifying** and **counting leukocytes** by **flow cytometry** - by eliminating erythrocyte influences and staining to identify at least 8 sub-populations.

AB EP 525398 A UPAB: 19931119

Prepn. of a specimen for **classifying** and **counting leukocytes** into at least 8 gps., i.e. **immature granulocytes** (2 gps.), erythroblasts, **basophils**, **eosinophils**, lymphocytes, monocytes and **neutrophils**, by assaying a single specimen with a flow cyotmeter, comprises (a) eliminating influences of erythrocytes from a haematological sample without changing the **leukocytes** morphologically by (i) fragmenting erythrocytes contained in the sample by adding an aq. soln. of

a low osmotic pressure comprising a buffer for maintaining the pH of the soln. within an acidic range to the sample and thus damaging the cell membranes of erythroblasts, (ii) adding to the mixt. a soln. comprising an

osmolarity compensating agent for maintaining the morphology of **leukocytes** unchanged and a buffer for neutralising the acid in the first aq. soln. and adjusting the pH to a level suitable for staining and (b) staining **leukocytes** in the sample with at least 4 dyes specified as follows: (i) Astrazon Yellow 34 capable of staining at least **basophils** and **immature granulocytes**, (ii) Neutral Red capable of staining at least **eosinophils**, (iii) a dye (I) capable of staining at least either or both of the nuclei and cytoplasm of **leukocytes** and (iv) a fluorochrome (II) capable of staining exclusively the nuclei of damaged cells.

USE/ADVANTAGE - By eliminating the influences of erythrocytes, **leukocytes** can be accurately **classified** and cell vol. can be accurately measured. The method allows **classification** of **leukocytes** into at least 8 gps. and further allows sepn. of blasts.

1/8

Dwg.1/8

ABEQ US 5264369 A UPAB: 19940120

Blood specimens are prepared by (A) eliminating influences of erythrocytes without damaging **leukocytes** morphology by (a) fragmentising the erythrocytes in the sample by adding an aq. soln. of low osmotic press contg. a buffer to maintain the mixt. obtd. at an acidic pH and to damage the cell membranes of erythroblasts only and (b) adding to the mixt. an osmology compensating soln. to maintain the morphology of the **leukocytes**, which also contains a buffer to adjust the pH to a level suitable for staining and (B) staining the blood corpuscles in the sample with (c) Astrazon Yellow 3G able to stain differentially at least **basophils** and **immature granulocytes**, (d) Neutral Red able to stain differentially at least **eosinophils**, (e) a dye able to stain differentially either or both of nuclei and cytoplasm of **leukocytes** and (f) a fluorochrome dye staining exclusively nuclei of the damaged erythroblasts. The dye for (Bc) is e.g.

Astra Violket, Cyanosine. The fluorochrome dye is e.g. propidium iodide.

USE - To classify and count blood gp. corpuscles into at least 8 gps., i.e. 2 comprising **immature granulocytes** gp. 1 and gp. 2, erythroblasts, **basophils**, **eosinophils**, lymphocytes, monocytes and **neutrophils** by assay of a single specimen.

Dwg. 1/8

ABEQ EP 525398 B UPAB: 19960719

A method for preparing a specimen for **classifying** and **counting leukocytes** into at least eight groups, namely, two each comprising **immature granulocytes**, one comprising erythroblasts, one comprising **basophils**, one comprising **eosinophils**, one comprising lymphocytes, one comprising monocytes and one comprising **neutrophils**, by assaying a single specimen with a **flow cytometer**, which comprises the following steps: (1) a step for eliminating influences of erythrocytes from a haematological sample without changing the **leukocytes** morphologically and comprises: (i) fragmenting erythrocytes contained in the haematological sample by adding a first aqueous solution of a low osmotic pressure comprising a buffer for maintaining the pH value of the solution within an acidic range to the haematological sample and thus damaging the cell membranes of erythroblasts; (ii) adding to the mixture obtained in (i) a second solution comprising an osmolarity compensating agent for maintaining the morphology of **leukocytes** unchanged and a buffer for neutralising the acid in the first aqueous solution and adjusting to a ph level suitable for staining; and (2) a step for staining **leukocytes** contained in the haematological sample with at least the four dyes specified below; (i) Astrazon Yellow 3G capable of staining at least **basophils** and **immature granulocytes**; (ii) Neutral Red capable of staining at least **eosinophils**; (iii) a dye capable of staining at least either or both of the nuclei and cytoplasm of **leukocytes**; and (iv) a fluorochrome capable of staining exclusively the nuclei of damaged cells.

Dwg.1/8

TT TT: **CLASSIFY COUNT LEUCOCYTE FLOW CYTOMETRY** ELIMINATE ERYTHROCYTE INFLUENCE STAIN IDENTIFY SUB POPULATION.

L74 ANSWER 8 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 1993-037829 [05] WPIDS

AB EP 525397 A UPAB: 19931119

Prepn. of a specimen for **classifying** and **counting leukocytes** into at least 3 gps. involving **immature granulocyte** by assaying a single specimen with a flow cyotmeter, which comprises opt. (a) eliminating influences of erythrocytes from a haematological sample without changing the **leukocytes** morphologically; (b) adjusting the pH of the haematological sample to a level suitable for staining, and (c) staining **leukocytes** contained in the haematological sample with at least 2 dyes specified as follows (i) Astrazon Yellow 3G capable of staining at least **basophils** and **immature granulocytes** and (ii) Neutral Red capable of staining at least **eosinophils**.

Step (a) may involve use of a dye selected from e.g. Astrazon Orange R, Astra Violet, Rhodamine 64, Rhodamine 19, Rhodamine B, Rhodamine 340, Pyronine B, Cyanosine, 3,3'-dimethylcarbocyanine iodide, 3,3'-dihexyloxacarbocyanine iodide, 3,6-bis(dimethylamino)-10-dodecylacridinium bromide, 7-benzylamino-4-nitrobenzoxadiazole, 7-fluoro-4-nitrbenzoxadiazole and Astrazon Red 6B.

USE/ADVANTAGE - Using the method it is possible to classify and count

immature granulocytes which cannot be achieved by known methods. When the influences of erythrocytes is eliminated, it is possible to classify and count at least 7 subpopulations of **leukocytes** in one sample.
1/32
Dwg.1/32

ABEQ US 5308772 A UPAB: 19940613
Method for **classifying** and **counting leukocytes** into at least 3 gps. includes **immature granulocytes** by assaying a single specimen with a **flow cytometer**, also comprises a step for adjusting a pH value of a haematological sample to a suitable level for staining, **leucocytes** are stained using Astrazon Yellow 3G to stain **basophils** and **immature granulocytes** and Neutral Red for staining **eosinophils**.
USE - For classifying and counting **immature granulocytes**.
Dwg.1/32

ABEQ EP 525397 B UPAB: 19970407
A method for preparing a specimen for **classifying** and **counting leukocytes** into at least three gps. involving **immature granulocytes** by assaying a single specimen with a **flow cytometer** which comprises the following steps (1) a step for adjusting the pH value of a hematological sample to a level suitable for staining, and (2) a step for staining **leukocytes** contained in the hematological sample with at least two dyes specified as follows: (i) Astrazon Yellow 3G capable of staining at least **basophils** and **immature granulocytes**, and (ii) Neutral Red capable of staining at least **eosinophils**.
Dwg.0/32

TI **Classifying** and **counting leukocytes** by **flow cytometry** - with staining with Astrazon Yellow 3G and Neutral Red to identify **immature granulocyte**.

AB EP 525397 A UPAB: 19931119
Prepn. of a specimen for **classifying** and **counting leukocytes** into at least 3 gps. involving **immature granulocyte** by assaying a single specimen with a flow cyotmeter, which comprises opt. (a) eliminating influences of erythrocytes from a haematological sample without changing the **leukocytes** morphologically; (b) adjusting the pH of the haematological sample to a level suitable for staining, and (c) staining **leukocytes** contained in the haematological sample with at least 2 dyes specified as follows (i) Astrazon Yellow 3G capable of staining at least **basophils** and **immature granulocytes** and (ii) Neutral Red capable of staining at least **eosinophils**.
Step (a) may involve use of a dye selected from e.g. Astrazon Orange R, Astra Violet, Rhodamine 64, Rhodamine 19, Rhodamine B, Rhodamine 340, Pyronine B, Cyanosine, 3,3'-dimethylcarbocyanine iodide, 3,3'-dihexyloxacarbocyanine iodide, 3,6-bis(dimethylamino)-10-dodecylacridinium bromide, 7-benzylamino-4-nitrobenzoxadiazole, 7-fluoro-4-nitrbenzoxadiazole and Astrazon Red 6B.
USE/ADVANTAGE - Using the method it is possible to classify and count **immature granulocytes** which cannot be achieved by known methods. When the influences of erythrocytes is eliminated, it is possible to classify and count at least 7 subpopulations of **leukocytes** in one sample.
1/32
Dwg.1/32

ABEQ US 5308772 A UPAB: 19940613
Method for **classifying** and **counting leukocytes** into at least 3 gps. includes **immature granulocytes** by assaying a single specimen with a **flow cytometer**, also comprises a step for adjusting a pH value of a haematological sample to a suitable level for staining, **leucocytes** are stained using Astrazon Yellow 3G to stain **basophils** and **immature granulocytes** and Neutral Red for staining **eosinophils**.
USE - For classifying and counting **immature granulocytes**.
Dwg.1/32

ABEQ EP 525397 B UPAB: 19970407
A method for preparing a specimen for **classifying** and **counting leukocytes** into at least three gps. involving **immature granulocytes** by assaying a single specimen with a **flow cytometer** which comprises the following steps (1) a step for adjusting the pH value of a hematological sample to a level suitable for staining, and (2) a step for staining **leukocytes** contained in the hematological sample with at least two dyes specified as follows: (i) Astrazon Yellow 3G capable of staining at least **basophils** and **immature granulocytes**, and (ii) Neutral Red capable of staining at least **eosinophils**.
Dwg.0/32

TT TT: **CLASSIFY COUNT LEUCOCYTE FLOW CYTOMETRY** STAIN YELLOW NEUTRAL RED IDENTIFY **IMMATURE GRANULOCYTE**.

L74 ANSWER 9 OF 10 MEDLINE DUPLICATE 3
93102713 Document Number: 93102713. Use of flow cytochemistry via the H*1 in FAB identification of acute leukaemias. Tsakona C P; Kinsey S E; Goldstone A H. (Department of Haematology, University College Hospital, London, UK..
) ACTA HAEMATOLOGICA, (1992) 88 (2-3) 72-7. Journal code: 0S8. ISSN: 0001-5792. Pub. country: Switzerland. Language: English.

AB Blood samples from 40 adult patients with untreated acute leukaemia were processed through the Technicon H*1 blood autoanalyser which gives a complete white cell differential count using flow cytochemistry and provides white cell cytograms as well. We examined the differences in the percentage differential counts and the white cell cytograms of various FAB types of acute leukaemia in an attempt to estimate the usefulness of this easily obtainable data for the identification of acute leukaemias. Differentiation of the 33 acute myeloid leukaemia (AML) cases from the 7 acute lymphoblastic leukaemia (ALL) cases was possible on the basis of lymphocyte percentage (AML mean 29.6 vs. ALL mean 67.1, $p < 0.01$), monocyte percentage (AML mean 12.5 vs. ALL mean 3.3, $p < 0.001$), mean peroxidase activity value (AML mean -12.6 vs. ALL mean -0.6, $p < 0.01$) and the absence of IG flag (circulating **immature granulocytes**) in ALL. Interestingly, the FAB subtypes of AML could be distinguished from each other on the basis of characteristic patterns of cell distribution in the peroxidase cytogram when the total white cell count was over 10 x 10(9)/l. Even with lower counts the differences were distinctive providing that circulating blasts were present.

AB Blood samples from 40 adult patients with untreated acute leukaemia were processed through the Technicon H*1 blood autoanalyser which gives a complete white cell differential count using flow cytochemistry and provides white cell cytograms as well. We examined the differences in the

percentage differential counts and the white cell cytograms of various FAB types of acute leukaemia in an attempt to estimate the usefulness of this easily obtainable data for the identification of acute leukaemias. Differentiation of the 33 acute myeloid leukaemia (AML) cases from the 7 acute lymphoblastic leukaemia (ALL) cases was possible on the basis of lymphocyte percentage (AML mean 29.6 vs. ALL mean 67.1, $p < 0.01$), monocyte percentage (AML mean 12.5 vs. ALL mean 3.3, $p < 0.001$), mean peroxidase activity value (AML mean -12.6 vs. ALL mean -0.6, $p < 0.01$) and the absence of IG flag (circulating **immature granulocytes**) in ALL. Interestingly, the FAB subtypes of AML could be distinguished from each other on the basis of characteristic patterns of cell distribution in the peroxidase cytogram when the total white cell count was over 10 x 10(9)/l. Even with lower counts the differences were distinctive providing that circulating blasts were present.

CT Check Tags: Human; Support, Non-U.S. Gov't
Adult
Autoanalysis: IS, instrumentation
***Flow Cytometry: IS, instrumentation**
Histocytochemistry
*Leukemia, Lymphocytic, Acute: BL, blood
*Leukemia, Lymphocytic, Acute: CL, classification
Leukemia, Lymphocytic, Acute: IM, immunology
*Leukemia, Myelocytic, Acute: BL, blood
*Leukemia, Myelocytic, Acute: CL, classification
Leukemia, Myelocytic, Acute: IM, immunology
***Leukocyte Count: IS, instrumentation**
Leukocytes, Mononuclear: PA, pathology
Lymphocytes: PA, pathology
Neutrophils: PA, pathology
Peroxidase: BL, blood

L74 ANSWER 10 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS
1988:261618 Document No.: BA86:862. DIFFERENTIAL **LEUKOCYTE COUNTING** WITH A NEW AUTOMATIC PARTICLE COUNTER. TVEDE K; HANSEN F R; WIMBERLEY P D; PARTOFT T. UROL. AFDELING H, KOBENHAVNS AMTS SYGEHUS, DK-2730 HERLEV.. UGESKR LAEG, (1987) 149 (52), 3535-3539. CODEN: UGLAAD. ISSN: 0041-5782. Language: Danish.

AB Assessment of automatic differential **counting** of the **leukocytes** was performed on the H1 apparatus (Technicon), employing optic **flow cytometry**. Differentiation of the various types of **leukocytes** is undertaken by peroxidase staining. The cells are subdivided into clusters depending on the peroxidase activity of the granules and their size. A shift to the left in the H1 apparatus is stated as **immature granulocytes** (IG) for LS (left shift) but more graduated subdivision of **immature granulocytes** is not obtained. These samples are submitted to microscopic examination. According to the criteria of selection, approximately 16% of the samples are submitted to microscopic examination daily. In the present investigation, two false negative samples were found but these were of lesser clinical significance (12% and 15% **granulocytes** with rod-shaped nuclei). A positive bias was found of approximately 10% for **leukocyte** concentrations within the normal range counted with the H1 apparatus. The **eosinophil granulocyte** concentration showed an unsystematic bias but this was without clinical significance. Introduction of the H1 apparatus has resulted in much greater precision and speed in analysis of differential **counting** of **leukocytes**.

TI DIFFERENTIAL **LEUKOCYTE COUNTING** WITH A NEW AUTOMATIC PARTICLE COUNTER.

AB Assessment of automatic differential **counting** of the **leukocytes** was performed on the H1 apparatus (Technicon), employing optic **flow cytometry**. Differentiation of the various types of **leukocytes** is undertaken by peroxidase staining. The cells are subdivided into clusters depending on the peroxidase activity of the granules and their size. A shift to the left in the H1 apparatus is stated as **immature granulocytes** (IG) for LS (left shift) but more graduated subdivision of **immature granulocytes** is not obtained. These samples are submitted to microscopic examination. According to the criteria of selection, approximately 16% of the samples are submitted to microscopic examination daily. In the present investigation, two false negative samples were found but these were of lesser clinical significance (12% and 15% **granulocytes** with rod-shaped nuclei). A positive bias was found of approximately 10% for **leukocyte** concentrations within the normal range counted with the H1 apparatus. The **eosinophil granulocyte** concentration showed an unsystematic bias but this was without clinical significance. Introduction of the H1 apparatus has resulted in much greater precision and speed in analysis of differential **counting** of **leukocytes**.

IT Miscellaneous Descriptors
FLOW CYTOMETRY PEROXIDASE STAINING PRECISION SPEED

=> s (light scatter? or light or light scatter/ct or turidimet?) and l64

L75 5 FILE MEDLINE
L76 0 FILE CAPLUS
L77 1 FILE BIOSIS
L78 3 FILE EMBASE
L79 4 FILE WPIDS
L80 0 FILE NTIS
L81 0 FILE COMPENDEX
L82 0 FILE INSPEC

TOTAL FOR ALL FILES
L83 13 (LIGHT SCATTER? OR LIGHT OR LIGHT SCATTER/CT OR TURIDIMET?) AND L64

=> s scatter?(2a)light and l64

L84 1 FILE MEDLINE
L85 0 FILE CAPLUS
L86 0 FILE BIOSIS
L87 1 FILE EMBASE
L88 0 FILE WPIDS
L89 0 FILE NTIS
L90 0 FILE COMPENDEX
L91 0 FILE INSPEC

TOTAL FOR ALL FILES
L92 2 SCATTER?(2A) LIGHT AND L64

=> s l92 or l83

L93 5 FILE MEDLINE

L94 0 FILE CAPLUS
L95 1 FILE BIOSIS
L96 3 FILE EMBASE
L97 4 FILE WPIDS
L98 0 FILE NTIS
L99 0 FILE COMPENDEX
L100 0 FILE INSPEC

TOTAL FOR ALL FILES
L101 13 L92 OR L83

=> dup rem l101

PROCESSING COMPLETED FOR L101
L102 11 DUP REM L101 (2 DUPLICATES REMOVED)

=> d cbib abs 1-11 hit

L102 ANSWER 1 OF 11 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
94165817 EMBASE Document No.: 1994165817. Electron microscopic findings of parallel tubular granules in a case of chronic **neutrophilic** leukaemia. Takemori N.; Hirai K.; Onodera R.; Saito N.; Namiki M.. Third Dept. of Internal Medicine, Asahikawa Medical College, 4-5, Nishikagura,Asahikawa 078, Japan. Journal of Clinical Pathology 47/4 (367-369) 1994.
ISSN: 0021-9746. CODEN: JCPAAK. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB A case of chronic **neutrophilic** leukaemia (CNL) is reported. The diagnosis was based on leucocytosis with mature **neutrophils**, a raised **leucocyte** alkaline phosphatase score, negative Philadelphia chromosome, and extensive infiltration of **neutrophils** in various differentiation stages into the bone marrow. When viewed by **light** microscopy, these **neutrophils** were almost normal in appearance, except for the presence of ring shaped nuclei and cytoplasmic vacuoles. Electron microscopy showed that **neutrophilic promyelocytes** and early **myelocytes** in the bone marrow frequently possessed unique rounded granules consisting of clustered parallel tubules, of 29-31 nm in diameter, and occasional amorphous electron dense material. These parallel tubules showed a hexagonal array; the granules were termed parallel tubular granules (PTGs). PTGs were positive for electron microscopic myeloperoxidase, and were seen exclusively in **neutrophilic promyelocytes** and early **myelocytes**. These findings suggest that PTGs represent certain primary granules peculiar to immature **neutrophils**. Their presence might also be helpful in diagnosing CNL.

TI Electron microscopic findings of parallel tubular granules in a case of chronic **neutrophilic** leukaemia.

AB A case of chronic **neutrophilic** leukaemia (CNL) is reported. The diagnosis was based on leucocytosis with mature **neutrophils**, a raised **leucocyte** alkaline phosphatase score, negative Philadelphia chromosome, and extensive infiltration of **neutrophils** in various differentiation stages into the bone marrow. When viewed by **light** microscopy, these **neutrophils** were almost normal in appearance, except for the presence of ring shaped nuclei and cytoplasmic vacuoles. Electron microscopy showed that **neutrophilic promyelocytes** and early **myelocytes** in the bone marrow frequently possessed unique rounded granules consisting of clustered parallel tubules, of 29-31 nm in diameter, and occasional amorphous electron dense material. These parallel tubules showed a hexagonal array; the granules were termed parallel tubular granules (PTGs). PTGs were

positive for electron microscopic myeloperoxidase, and were seen exclusively in **neutrophilic promyelocytes** and early **myelocytes**. These findings suggest that PTGs represent certain primary granules peculiar to immature **neutrophils**. Their presence might also be helpful in diagnosing CNL.

CT Medical Descriptors:
*chronic myeloid leukemia: DI, diagnosis
*chronic myeloid leukemia: DT, drug therapy
aged
article
case report
cell granule
human
human cell
leukocytosis
male
thrombocytopenia: DT, drug therapy
thrombocytopenia: SI, side effect
ultrastructure
Drug Descriptors:
*carboquone: DT, drug therapy
*carboquone: AE, adverse drug reaction
*hydroxyurea: DT, drug therapy
*hydroxyurea: AE, adverse drug reaction
cepharanthine: DT, drug therapy

L102 ANSWER 2 OF 11 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
93030676 EMBASE Document No.: 1993030676. Evaluation of the Coulter STKS five-part differential. Cornbleet P.J.; Myrick D.; Levy R.. Clinical Laboratories, Stanford University Medical Center, 300 Pasteur Dr.,Stanford, CA 94305, United States. American Journal of Clinical Pathology 99/1 (72-81) 1993.
ISSN: 0002-9173. CODEN: AJCPAI. Pub. Country: United States. Language: English. Summary Language: English.

AB The authors evaluated the Coulter STKS (Coulter Corp., Hialeah, FL) five-part differential in a tertiary-care hospital using samples with a broad range of distributional and morphologic abnormalities. Particular attention was given to the performance of the instrument-generated suspect flags that occur as an aid to identify samples with abnormal **leukocytes**. A morphologically abnormal, or positive, blood smear was defined by the presence of any blasts, malignant lymphoid cells, grossly dysplastic **neutrophils**, nucleated red blood cells (nRBC), platelet clumps, or reactive lymphocytes of more than 5%. The presence of any white blood cell-related suspect flag, except for **Immature Granulocyte**/Bands (i.e., Blasts, Variant Lymph, NRBC, Platelet Clumps, Review Slide, or WBC *R), was considered to be a positive instrument result. The STKS showed excellent quantitative results for the WBC differential compared with the manual differential when these 'morphologic abnormalities' were absent in a 400-cell manual differential or low in numbers (.ltoreq. 5%). Specificity of these non-**immature granulocyte**/band suspect flags was good, with a false-positive rate of only 11.7%. Overall sensitivity in 113 samples with morphologic abnormalities was 67.3%. Sensitivity to detection of .gtoreq. 1% abnormal WBCs or .gtoreq. 1 nRBC/100 WBCs (a subset of 78 samples) was 80.8%. Sensitivity to detection of more than 5% abnormal WBCs or more than 5 nRBC/100 WBCs (a subset of 53 samples) was 84.9%. The primary deficiency was the inability of the STKS to flag samples with lymphoma cells, lymphoid blasts, or more than 5% reactive lymphocytes.

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part differential in a tertiary-care hospital using samples with a broad range of distributional and morphologic abnormalities. Particular attention was given to the performance of the instrument-generated suspect flags that occur as an aid to identify samples with abnormal **leukocytes**. A morphologically abnormal, or positive, blood smear was defined by the presence of any blasts, malignant lymphoid cells, grossly dysplastic **neutrophils**, nucleated red blood cells (nRBC), platelet clumps, or reactive lymphocytes of more than 5%. The presence of any white blood cell-related suspect flag, except for **Immature Granulocyte**/Bands (i.e., Blasts, Variant Lymph, NRBC, Platelet Clumps, Review Slide, or WBC *R), was considered to be a positive instrument result. The STKS showed excellent quantitative results for the WBC differential compared with the manual differential when these 'morphologic abnormalities' were absent in a 400-cell manual differential or low in numbers (.ltoreq. 5%). Specificity of these non-**immature granulocyte**/band suspect flags was good, with a false-positive rate of only 11.7%. Overall sensitivity in 113 samples with morphologic abnormalities was 67.3%. Sensitivity to detection of .gtoreq. 1% abnormal WBCs or .gtoreq. 1 nRBC/100 WBCs (a subset of 78 samples) was 80.8%. Sensitivity to detection of more than 5% abnormal WBCs or more than 5 nRBC/100 WBCs (a subset of 53 samples) was 84.9%. The primary deficiency was the inability of the STKS to flag samples with lymphoma cells, lymphoid blasts, or more than 5% reactive lymphocytes.

CT Medical Descriptors:
*laboratory automation
***leukocyte differential count**
article
basophil
blood smear
cell structure
diagnostic accuracy
eosinophil
laser
light scattering
lymphoid cell
neutrophil
priority journal

L102 ANSWER 3 OF 11 MEDLINE
92213895 Document Number: 92213895. A **light** and electron microscopic study of changes in blood and bone marrow in acute hemorrhagic Trypanosoma vivax infection in calves. Anosa V O; Logan-Henfrey L L; Shaw M K. (International Laboratory for Research on Animal Diseases, Nairobi, Kenya..) VETERINARY PATHOLOGY, (1992 Jan) 29 (1) 33-45. Journal code: XBQ. ISSN: 0300-9858. Pub. country: United States. Language: English.

AB Eleven 6-month-old calves were tsetse fly challenged with a stock of Trypanosoma vivax (IL 2337) that causes hemorrhagic infection. The calves were randomly euthanatized every 4 to 6 days; two other calves served as controls. Peripheral blood changes included anemia, thrombocytopenia, and an initial leukopenia. Later in the course of infection, leukocytosis associated with lymphocytosis and neutropenia developed. Moderate reticulocytosis (highest mean count 3.6 +/- 3.7%, maximum count 9.4%) accompanied the first wave of parasitemia, but poor response (highest mean 0.4 +/- 0.0%) occurred during the second wave, despite the persistence of severe anemia. **Light** microscopic examination of bone marrow samples showed a drop in the myeloid: erythroid ratio with a decrease in **granulocytes**, particularly **metamyelocytes**, bands, and

segmenters. Increase in **lymphocyte counts** corresponded with the appearance of lymphoid nodules within the marrow. Megakaryocytic volume increased significantly in infected animals, and some megakaryocytes showed emperipolesis of red cells, **neutrophils**, and lymphocytes. Transmission electron microscopic examination of the bone marrow revealed that trypanosomes had crossed the sinusoidal endothelium into the hematopoietic compartment as early as the second day of parasitemia. Macrophages proliferated in the bone marrow; and from the second day of parasitemia until the end of the experimental infection, on day 46, the macrophages had phagocytosed normoblasts, **eosinophil** and **neutrophil myelocytes**, **metamyelocytes**, bands, and segmenters, as well as reticulocytes, erythrocytes, and thrombocytes. Therefore, dyserythropoiesis and dysgranulocytopoiesis were responsible, in part, for the observed anemia and granulocytopenia, respectively.

TI A **light** and electron microscopic study of changes in blood and bone marrow in acute hemorrhagic Trypanosoma vivax infection in calves.

AB Eleven 6-month-old calves were tsetse fly challenged with a stock of Trypanosoma vivax (IL 2337) that causes hemorrhagic infection. The calves were randomly euthanatized every 4 to 6 days; two other calves served as controls. Peripheral blood changes included anemia, thrombocytopenia, and an initial leukopenia. Later in the course of infection, leukocytosis associated with lymphocytosis and neutropenia developed. Moderate reticulocytosis (highest mean count 3.6 +/- 3.7%, maximum count 9.4%) accompanied the first wave of parasitemia, but poor response (highest mean 0.4 +/- 0.0%) occurred during the second wave, despite the persistence of severe anemia. **Light** microscopic examination of bone marrow samples showed a drop in the myeloid: erythroid ratio with a decrease in **granulocytes**, particularly **metamyelocytes**, bands, and segmenters. Increase in **lymphocyte counts** corresponded with the appearance of lymphoid nodules within the marrow. Megakaryocytic volume increased significantly in infected animals, and some megakaryocytes showed emperipolesis of red cells, **neutrophils**, and lymphocytes. Transmission electron microscopic examination of the bone marrow revealed that trypanosomes had crossed the sinusoidal endothelium into the hematopoietic compartment as early as the second day of parasitemia. Macrophages proliferated in the bone marrow; and from the second day of parasitemia until the end of the experimental infection, on day 46, the macrophages had phagocytosed normoblasts, **eosinophil** and **neutrophil myelocytes**, **metamyelocytes**, bands, and segmenters, as well as reticulocytes, erythrocytes, and thrombocytes. Therefore, dyserythropoiesis and dysgranulocytopoiesis were responsible, in part, for the observed anemia and granulocytopenia, respectively.

CT Check Tags: Animal
Acute Disease
*Bone Marrow: PA, pathology
Bone Marrow: UL, ultrastructure
Cattle
Cell Count: VE, veterinary
Erythrocyte Count: VE, veterinary
Hematocrit: VE, veterinary
Hemorrhage: BL, blood
Hemorrhage: PA, pathology
*Hemorrhage: VE, veterinary
Leukocyte Count: VE, veterinary
Microscopy, Electron
Platelet Count: VE, veterinary

Reticulocytes: CY, cytology
*Trypanosoma vivax
Trypanosomiasis, African: BL, blood
*Trypanosomiasis, African: PA, pathology
Trypanosomiasis, Bovine: BL, blood
*Trypanosomiasis, Bovine: PA, pathology

L102 ANSWER 4 OF 11 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1985-302955 [48] WPIDS
AB WO 8505179 A UPAB: 19930925
Identification, differentiation and ennumeration of sub-populations of human blood lymphocytes comprises (a) metochromatically staining a human blood-contg. specimen in an aq. supravital fixative-free environment with a basic quaternary organic dye comprising basic orange 21, (b) subjecting the dyed specimen to at least 1 **light** energy stimulus based upon absorbence and/or fluorescence.

The following sub-populations are differentiated and identified as follows: (i) T-helpen cells under absorbence by their relatively darker yellow nucleus and cytoplasm than present in the lighter yellow B-cells and prominent nuclear chromatin aggregates in the cells, which cells are smaller than B-cells and under fluorescence displaying a darker blue-green fluorescent nucleus and a lighter blue-green fluorescent cytoplasm; (ii) T-suppressor celles which are similar in size and colouration to the T-helper cells are distinguished under absorbence **light** by the presence of a relatively few associated small red granules in the cytoplasm and under fluorescence by similar but fluorescent blue-green nucleus and cytoplasm, and the associated small granules characterised by a yellow fluorescence and (iii) natural killer cells or NK cells are identified as overall larger than either the B-cells or T-cells, lighter yellow and larger nucleus and cytoplasm, displaying chromatin aggregates in the nucleus, but further distinguished under absorbence by the larger yellow fluorescent cytoplasm area contg. a larger number of aggregated fluorescent red granules occupying a larger overall area than the T-suppressor cells and the NK cells under fluorescence display a fluorescent green nucleus and cytoplasm and large aggregated granules in the cytoplasm fluoresce a bright yellow.

ADVANTAGE - The dye makes it possible to specularly identify the cells qualitatively and quantitatively using the same dyestuff in the same supravital, fixative free analysis.

0/1

ABEQ DE 3208629 C UPAB: 19930925
Microscopy for supravital blood analysis comprises treating a blood sample with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an optical counter.

USE - Identification and **counting** of mature **leucocytes, immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral, **eosinophilic** and **basophilic** cells for diagnosis.

ABEQ DE 3304795 C UPAB: 19930925
Microscopic analysis for the identification, counting and investigation of cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with

light of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes, myelocytes, metamyelocytes, neutrophilic, eosinophilic** and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ EP 179107 B UPAB: 19930925

A method of identification, differentiation and enumeration of sub-populations of human blood lymphocytes hitherto selectively identified by means of monoclonal antibody affinity, which comprises: metachromatically staining a human blood containing specimen in an aqueous supravital fixative-free environment with a basic quanternary organic dyestuff comprising basic orange 21; subjecting the dyed specimen to at least one **light** energy stimulus based upon absorbence and/or fluorescence and thereafter differentiating and identifying the following sub-populations as follows: T-helper cells under absorbence by their relatively darker yellow nucleus and cytoplasm than present in the lighter yellow B-cells and prominent nuclear chromatin aggregates in said cells which cells are relatively smaller than B-cells and under fluorescence displaying a darker blue-green fluorescent nucleus and a noticeably lighter blue-green fluorescent cytoplasm; the T-suppressor cells which are generally similar in size and coloration to the T-helper cells are distinguished under adsorbance by the presence of a relatively few associated small red granules in the cytoplasm and under fluorescence by similar but fluorescent blue-green nucleus and cytoplasm, and the associated small granules alternatively characterized by a yellow fluorescence; and, natural killer cells abbreviated as NK cells are further identified by being overall larger than either the above B-cells and the above T-cells, somewhat lighter yellow and having a larger nucleus and cytoplasm, displaying chromatin aggregates in the nucleus, but further distinguished under absorbence by the larger yellow cytoplasm area containing a generally larger number of aggregated red granules occupying a larger overall area than the T-suppressor cells and which said NK cells under fluorescence display a fluorecent green nucleus and cytoplasm and wherein large aggregated granules in the cytoplasm fluoresce a bright yellow, thereby distinguishing e

ABEQ US 4615878 A UPAB: 19930925

Qualitative and quantitative analysis of human blood specimens under supravital conditions to determine subpopulations of lymphocytes consisting of B-cells, T-helper cells, T-suppressor cells and Natural Killer (NK) cells comprises staining a fixative-free specimen with aq. soln. contg. up to 1% cationic dye Basic Orange 21.

The dyed specimen is exposed to white or fluorescent **light** and the subpopulations are differentiated, identified and enumerated by comparison of the size, shape, colour and component differentials within the nucleus and cytoplasm of each cell. This includes the presence or absence of and the relative size, number and characteristic colour of granules observable in the cytoplasm and the characteristic colour differentials among the subpopulations in their respective nuclei.

ADVANTAGE - A single supravital dye strain is used. The dye is of

relatively low toxicity and is fast acting.

AB WO 8505179 A UPAB: 19930925

Identification, differentiation and ennumeration of sub-populations of human blood lymphocytes comprises (a) metochromatically staining a human blood-contg. specimen in an aq. supravital fixative-free environment with a basic quaternary organic dye comprising basic orange 21, (b) subjecting the dyed specimen to at least 1 **light** energy stimulus based upon absorbence and/or fluorescence.

The following sub-populations are differentiated and identified as follows: (i) T-helpen cells under absorbence by their relatively darker yellow nucleus and cytoplasm than present in the lighter yellow B-cells and prominent nuclear chromatin aggregates in the cells, which cells are smaller than B-cells and under fluorescence displaying a darker blue-green fluorescent nucleus and a lighter blue-green fluorescent cytoplasm; (ii) T-suppressor celles which are similar in size and colouration to the T-helper cells are distinguished under absorbence **light** by the presence of a relatively few associated small red granules in the cytoplasm and under fluorescence by similar but fluorescent blue-green nucleus and cytoplasm, and the associated small granules characterised by a yellow fluorescence and (iii) natural killer cells or NK cells are identified as overall larger than either the B-cells or T-cells, lighter yellow and larger nucleus and cytoplasm, displaying chromatin aggregates in the nucleus, but further distinguished under absorbence by the larger yellow fluorescent cytoplasm area contg. a larger number of aggregated fluorescent red granules occupying a larger overall area than the T-suppressor cells and the NK cells under fluorescence display a fluorescent green nucleus and cytoplasm and large aggregated granules in the cytoplasm fluoresce a bright yellow.

ADVANTAGE - The dye makes it possible to specularly identify the cells qualitatively and quantitatively using the same dyestuff in the same supravital, fixative free analysis.

0/1

ABEQ DE 3208629 C UPAB: 19930925

Microscopy for supravital blood analysis comprises treating a blood sample with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an optical counter.

USE - Identification and **counting** of mature **leucocytes, immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral, **eosinophilic** and **basophilic** cells for diagnosis.

ABEQ DE 3304795 C UPAB: 19930925

Microscopic analysis for the identification, counting and investigation of cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with **light** of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes, myelocytes, metamyelocytes, neutrophilic, eosinophilic** and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ EP 179107 B UPAB: 19930925

A method of identification, differentiation and enumeration of sub-populations of human blood lymphocytes hitherto selectively identified by means of monoclonal antibody affinity, which comprises: metachromatically staining a human blood containing specimen in an aqueous supravital fixative-free environment with a basic quanternary organic dyestuff comprising basic orange 21; subjecting the dyed specimen to at least one **light** energy stimulus based upon absorbence and/or fluorescence and thereafter differentiating and identifying the following sub-populations as follows: T-helper cells under absorbence by their relatively darker yellow nucleus and cytoplasm than present in the lighter yellow B-cells and prominent nuclear chromatin aggregates in said cells which cells are relatively smaller than B-cells and under fluorescence displaying a darker blue-green fluorescent nucleus and a noticeably lighter blue-green fluorescent cytoplasm; the T-suppressor cells which are generally similar in size and coloration to the T-helper cells are distinguished under adsorbance by the presence of a relatively few associated small red granules in the cytoplasm and under fluorescence by similar but fluorescent blue-green nucleus and cytoplasm, and the associated small granules alternatively characterized by a yellow fluorescence; and, natural killer cells abbreviated as NK cells are further identified by being overall larger than either the above B-cells and the above T-cells, somewhat lighter yellow and having a larger nucleus and cytoplasm, displaying chromatin aggregates in the nucleus, but further distinguished under absorbence by the larger yellow cytoplasm area containing a generally larger number of aggregated red granules occupying a larger overall area than the T-suppressor cells and which said NK cells under fluorescence display a fluorecent green nucleus and cytoplasm and wherein large aggregated granules in the cytoplasm fluoresce a bright yellow, thereby distinguishing e

ABEQ US 4615878 A UPAB: 19930925

Qualitative and quantitative analysis of human blood specimens under supravital conditions to determine subpopulations of lymphocytes consisting of B-cells, T-helper cells, T-suppressor cells and Natural Killer (NK) cells comprises staining a fixative-free specimen with aq. soln. contg. up to 1% cationic dye Basic Orange 21.

The dyed specimen is exposed to white or fluorescent **light** and the subpopulations are differentiated, identified and enumerated by comparison of the size, shape, colour and component differentials within the nucleus and cytoplasm of each cell. This includes the presence or absence of and the relative size, number and characteristic colour of granules observable in the cytoplasm and the characteristic colour differentials among the subpopulations in their respective nuclei.

ADVANTAGE - A single supravital dye strain is used. The dye is of relatively low toxicity and is fast acting.

L102 ANSWER 5 OF 11 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 1983-771982 [39] WPIDS

AB DE 3304795 A UPAB: 19930925

Microscopic analysis of human blood **leukocytes** and lymphocytes and the development stages of **neutrophilic** granulocytic cells is effected by staining the sample with a fixer-free aq. soln. of a basic dye

(I) and exposing the stained sample to an emission wave energy (sic) to excite fluorescence, a device supplying emitted fluorescent **light** (sic) being provided to differentiate monocytes from other blood cells. (I) is basic orange 21, basic red 13, 36 or 49, or basic violet 7, 15, 16, 36, 39 or 40.

Specified blood cells include **promyelocytes**, **myelocytes, metamyelocytes, band cells** , **neutrophils, eosinophils, basophils**, monocytes, B and T cells and other **leukocytes**, all giving a characteristic absorption or fluorescence staining pattern.

0/1

ABEQ DE 3208629 C UPAB: 19930925

Microscopy for supravital blood analysis comprises treating a blood sample with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an optical counter.

USE - Identification and **counting** of mature **leucocytes, immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral, **eosinophilic** and **basophilic** cells for diagnosis.

ABEQ DE 3304795 C UPAB: 19930925

Microscopic analysis for the identification, counting and investigation of cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with **light** of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes, myelocytes, metamyelocytes, neutrophilic, eosinophilic** and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ GB 2116712 B UPAB: 19930925

A method of identifying, differentiating and enumerating **leukocyte** and lymphocyte species and/or the developmental stages of **neutrophilic** and granulocytic cells present in a supravital human blood specimen, which comprises staining the specimen in an aqueous fixative free environment with the dyestuff basic orange 21, and observing the stained cells whilst under exposure to radiant energy effective to cause fluorescence of the stained cells.

ABEQ US 4500509 A UPAB: 19930925

Analysis of human blood cells in a donor specimen in a fixative-free aq. environment comprises staining the specimen with an aq. soln. of basic cationic quat. organic dyestuff chosen from basic orange.21, basic red 13, 36 or 49, basic violet 7, 15, 16, 36, 39 or 40 and carbocyanine K-5. The dye stained specimen is subjected to emissive wave energy to stimulate fluoresence of the dye exposed cells and the emitted fluoresence **light** is used to differentiate monocytes from all other cells.

USE/ADVANTAGE - For identification, enumeration, and study of monocytes in the specimen. Under bimodal **light** sources, each species e.g. myeloblasts, **neutrophils**, T-lymphocytes, etc. can be observed individually.

AB DE 3304795 A UPAB: 19930925
Microscopic analysis of human blood **leukocytes** and lymphocytes and the development stages of **neutrophilic** granulocytic cells is effected by staining the sample with a fixer-free aq. soln. of a basic dye
(I) and exposing the stained sample to an emission wave energy (sic) to excite fluorescence, a device supplying emitted fluorescent **light** (sic) being provided to differentiate monocytes from other blood cells.
(I) is basic orange 21, basic red 13, 36 or 49, or basic violet 7, 15, 16,
36, 39 or 40.

Specified blood cells include **promyelocytes**, **myelocytes**, **metamyelocytes**, **band cells** , **neutrophils**, **eosinophils**, **basophils**, monocytes, B and T cells and other **leukocytes**, all giving a characteristic absorption or fluorescence staining pattern.
0/1

ABEQ DE 3208629 C UPAB: 19930925
Microscopy for supravital blood analysis comprises treating a blood sample
with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an
optical counter.

USE - Identification and **counting** of mature **leucocytes**, **immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral,
eosinophilic and **basophilic** cells for diagnosis.

ABEQ DE 3304795 C UPAB: 19930925
Microscopic analysis for the identification, counting and investigation of
cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with **light** of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes**, **myelocytes**, **metamyelocytes**, **neutrophilic**, **eosinophilic** and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ GB 2116712 B UPAB: 19930925
A method of identifying, differentiating and enumerating **leukocyte** and lymphocyte species and/or the developmental stages of **neutrophilic** and granulocytic cells present in a supravital human blood specimen, which comprises staining the specimen in an aqueous fixative free environment with the dyestuff basic orange 21, and observing
the stained cells whilst under exposure to radiant energy effective to cause fluorescence of the stained cells.

ABEQ US 4500509 A UPAB: 19930925
Analysis of human blood cells in a donor specimen in a fixative-free aq. environment comprises staining the specimen with an aq. soln. of basic cationic quat. organic dyestuff chosen from basic orange.21, basic red 13, 36 or 49, basic violet 7, 15, 16, 36, 39 or 40 and carbocyanine K-5. The dye stained specimen is subjected to emissive wave energy to stimulate fluoresence of the dye exposed cells and the emitted fluoresence **light** is used to differentiate monocytes from all other cells.

USE/ADVANTAGE - For identification, enumeration, and study of monocytes in the specimen. Under bimodal **light** sources, each species e.g. myeloblasts, **neutrophils**, T-lymphocytes, etc. can be observed individually.

L102 ANSWER 6 OF 11 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1982-81589E [39] WPIDS
AB DE 3208629 A UPAB: 19930915
In a new microscopic procedure for the supravital analysis of **leukocytes** and lymphocytes of normal or pathological human blood (including leukaemic lymphoblasts potentially present in a donor blood specimen) in a fixative-free aq. medium, the blood specimen or a fraction thereof is subjected to the action of the dye Basic Orange, the dye being metachromatically and differentially absorbed by each stained species present in the specimen.

Used for differentiation, identification and counting of various types of lymphocytes and **leukocytes** in medical research and in the diagnosis and prognosis of various disease states. Fast and accurate procedure is provided utilising a single, pure dye without fixatives.

ABEQ GB 2095402 B UPAB: 19930915
A method of differentially staining human blood cell species selected from one or more of the following: myeloblasts, **promyelocytes**, **myelocytes**, **metamyelocytes**, bands, B-cells and T-cells and which may be present in a supravital human blood sample for the purpose of optical differentiation and t determination of said species, which comprises contacting the sample, or a fraction thereof, in a fixative-free aq. environment with the basic quaternary organic cationic dyestuff hereinbefore identified and named as Basic organe No.21 (Spectral Curve 7), thereby to differentially stain such of said blood cell species as may be present in said blood sample or fraction, and thereafter optically evaluating the stained sample for the presence or absence of at least one of the said selected species differentiated from other species present in the sample by the sorption and differential staning of that species by said dyestuff.

ABEQ DE 3304795 C UPAB: 19930915
Microscopic analysis for the identification, counting and investigation of cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with **light** of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes**, **myelocytes**, **metamyelocytes**, **neutrophilic**, **eosinophilic** and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the

dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ DE 3208629 C UPAB: 19930915

Microscopy for supravital blood analysis comprises treating a blood sample with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an optical counter.

USE - Identification and **counting** of mature **leucocytes**, **immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral, **eosinophilic** and **basophilic** cells for diagnosis.

TI Microscopic supravital analysis of **leukocytes** and lymphocytes - in fixative-free aq. medium by differential meta chromatic staining with basic orange.

AB DE 3208629 A UPAB: 19930915

In a new microscopic procedure for the supravital analysis of **leukocytes** and lymphocytes of normal or pathological human blood (including leukaemic lymphoblasts potentially present in a donor blood specimen) in a fixative-free aq. medium, the blood specimen or a fraction thereof is subjected to the action of the dye Basic Orange, the dye being metachromatically and differentially absorbed by each stained species present in the specimen.

Used for differentiation, identification and counting of various types of lymphocytes and **leukocytes** in medical research and in the diagnosis and prognosis of various disease states. Fast and accurate procedure is provided utilising a single, pure dye without fixatives.

ABEQ GB 2095402 B UPAB: 19930915

A method of differentially staining human blood cell species selected from one or more of the following: myeloblasts, **promyelocytes**, **myelocytes**, **metamyelocytes**, bands, B-cells and T-cells and which may be present in a supravital human blood sample for the purpose of optical differentiation and t determination of said species, which comprises contacting the sample, or a fraction thereof, in a fixative-free aq. environment with the basic quaternary organic cationic dyestuff hereinbefore identified and named as Basic organe No.21 (Spectral Curve 7), thereby to differentially stain such of said blood cell species as may be present in said blood sample or fraction, and thereafter optically evaluating the stained sample for the presence or absence of at least one of the said selected species differentiated from other species present in the sample by the sorption and differential staning of that species by said dyestuff.

ABEQ DE 3304795 C UPAB: 19930915

Microscopic analysis for the identification, counting and investigation of cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with **light** of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes**, **myelocytes**, **metamyelocytes**, **neutrophilic**, **eosinophilic**

and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ DE 3208629 C UPAB: 19930915

Microscopy for supravital blood analysis comprises treating a blood sample with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an optical counter.

USE - Identification and **counting** of mature **leucocytes**, **immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral, **eosinophilic** and **basophilic** cells for diagnosis.

TT TT: MICROSCOPIC ANALYSE **LEUCOCYTE** LYMPHOCYTE FIX FREE AQUEOUS MEDIUM DIFFERENTIAL META CHROMATIC STAIN BASIC ORANGE.

L102 ANSWER 7 OF 11 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 1981-78187D [43] WPIDS

AB FR 2478317 A UPAB: 19930915

The various categories of **leucocytes** are differentiated, identified, compared and counted in a live blood sample by treating the sample, which is free of fixing agents, with at least one organic, cationic (quaternary) metachromatic dye (I) in aq. soln. (I) is able to dye **leucocytes** at normal blood temp., inducing a spectral difference (reflected visible spectrum) between the various categories. There is an order of magnitude difference between the reflected **light** spectrum of **leucocytes** which have absorbed (I) and those of the same category which have not absorbed (I).

Categories which can be differentiated are polymorphonuclear **leucocytes**, **eosinophiles**, **basophiles**, lymphocytes and monocytes.

All five categories can be determined precisely and rapidly without complicated manipulation, to establish the **leucocyte** formula.

ABEQ GB 2074749 B UPAB: 19930915

A method of differentially staining **leukocyte** species present in a supravital human blood sample for the purpose of optical differentiation and determination of said species, which comprises contacting the sample in a fixative-free aqueous environment and at a temperature in the range 21-40 deg. C with a basic quaternary organic cationic dyestuff or a mixture of two or more thereof, being a dyestuff or dyestuff mixture sorbable metachromatically by at least one of the **leukocyte** species present in the sample and serving to differentiate that species visually from the other **leukocyte** species also present, said dyestuff or dyestuff mixture further being characterised by the specific ability to stain monocytes metachromatically in an aqueous fixative-free environment at temperatures in the range 37-40 deg. C.

ABEQ DE 3109252 C UPAB: 19930915

Determination of individual **leucocyte** types by selective metachromatic dye absorption comprises addn. of one or more cationic quat. dyes to human blood samples, when at least some **leucocyte** types are metachromatically coloured and readily counted. Suitable dyes are condor wood blue, borrel blue, thiocyanate blue, toluylene blue, night blue, pure plum, Hofmann's violet, basic orange 21, basic red 13, basic violet 16 and carbocyanine K-5.

USE - The process is applicable to mature **leucocytes**, e.g.

neutrophilic, **eosinophilic**, **basophilic** and
lumphocytic monocytes, esp. for clinical diagnosis.

ABEQ DE 3304795 C UPAB: 19930915

Microscopic analysis for the identification, counting and investigation of
cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with **light** of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes**, **myelocytes**, **metamyelocytes**, **neutrophilic**, **eosinophilic** and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ DE 3208629 C UPAB: 19930915

Microscopy for supravital blood analysis comprises treating a blood sample with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an optical counter.

USE - Identification and **counting** of mature **leucocytes**, **immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral, **eosinophilic** and **basophilic** cells for diagnosis.

TI Determination of different **leucocyte** categories - by staining with meta chromatic cationic dyes.

AB FR 2478317 A UPAB: 19930915

The various categories of **leucocytes** are differentiated, identified, compared and counted in a live blood sample by treating the sample, which is free of fixing agents, with at least one organic, cationic (quaternary) metachromatic dye (I) in aq. soln. (I) is able to dye **leucocytes** at normal blood temp., inducing a spectral difference (reflected visible spectrum) between the various categories. There is an order of magnitude difference between the reflected **light** spectrum of **leucocytes** which have absorbed (I) and those of the same category which have not absorbed (I).

Categories which can be differentiated are polymorphonuclear **leucocytes**, **eosinophiles**, **basophiles**, lymphocytes and monocytes.

All five categories can be determined precisely and rapidly without complicated manipulation, to establish the **leucocyte** formula.

ABEQ GB 2074749 B UPAB: 19930915

A method of differentially staining **leukocyte** species present in a supravital human blood sample for the purpose of optical differentiation and determination of said species, which comprises contacting the sample in a fixative-free aqueous environment and at a temperature in the range 21-40 deg. C with a basic quaternary organic cationic dyestuff or a mixture of two or more thereof, being a dyestuff or dyestuff mixture sorbable metachromatically by at least one of the **leukocyte** species present in the sample and serving to differentiate that species visually from the other **leukocyte** species also present, said

dyestuff or dyestuff mixture further being characterised by the specific ability to stain monocytes metachromatically in an aqueous fixative-free environment at temperatures in the range 37-40 deg. C.

ABEQ DE 3109252 C UPAB: 19930915

Determination of individual **leucocyte** types by selective metachromatic dye absorption comprises addn. of one or more cationic quat.

dyes to human blood samples, when at least some **leucocyte** types are metachromatically coloured and readily counted. Suitable dyes are condor wood blue, borrel blue, thiocyanate blue, toluylene blue, night blue, pure plum, Hofmann's violet, basic orange 21, basic red 13, basic violet 16 and carbocyanine K-5.

USE - The process is applicable to mature **leucocytes**, e.g. **neutrophilic**, **eosinophilic**, **basophilic** and lumphocytic monocytes, esp. for clinical diagnosis.

ABEQ DE 3304795 C UPAB: 19930915

Microscopic analysis for the identification, counting and investigation of

cell species from a supravital donor probe comprises treating the probe with Basic Orange 21 as a metachromic fluorochromic dye in an aq. medium free from fixative, and sepg. the various cell types according to the pattern observed when the cells are simultaneously illuminated with **light** of a wavelength causing the dye to fluoresce, and white **light**. The observation is made optically, and the fluorescence and absorption patterns of nucleus, cytoplasm etc. are both observed. The cells are blood **leucocytes** and lymphocytes and various development stages of **neutrophilic** and granulocytic cells, esp. myeloblasts, **promyelocytes**, **myelocytes**, **metamyelocytes**, **neutrophilic**, **eosinophilic** and/or **basophilic** cells, and B- and T-lymphocytes.

USE/ADVANTAGE - The absence of fixative (e.g. formalin) improves the dye absorption and reduces the time for the test. The fluorescent **light** is laser and the simultaneous laser and white **light** improves the ease of distinguishing the patterns.

ABEQ DE 3208629 C UPAB: 19930915

Microscopy for supravital blood analysis comprises treating a blood sample

with Basic Orange 21, to absorb dye metachromatically and to different extents by the components and observing sample under a microscope or by an

optical counter.

USE - Identification and **counting** of mature **leucocytes**, **immature granulocyte** cells (e.g. myeloblasts,) myeloid cells, aggregates, B- and T-lymphocytes and neutral,

eosinophilic and **basophilic** cells for diagnosis.

TT TT: DETERMINE **LEUCOCYTE** CATEGORY STAIN META CHROMATIC CATION DYE.

L102 ANSWER 8 OF 11 MEDLINE

80127415 Document Number: 80127415. **Neutrophil** marrow in chronic benign idiopathic neutropenia. Dancey J T; Brubaker L H. AMERICAN JOURNAL OF MEDICINE, (1980 Feb) 68 (2) 251-4. Journal code: 3JU. ISSN: 0002-9343.

Pub. country: United States. Language: English.

AB Quantitative studies of **neutrophil** marrow were carried out in 10 patients with chronic neutropenia (60 to 1,970 cells/microliter) with no other abnormalities and no serious infections. **Neutrophil** marrow cellularity was determined from **neutrophil**-normoblast ratios in marrow sections and ferrokinetic estimation of normoblasts. The results were interpreted in the **light** of 95 per cent confidence limits

previously observed in 13 normal volunteer subjects. Three distinct **neutrophil** marrow profiles were determined by the numbers of **promyelocytes** and **myelocytes** and of segmented marrow cells. Tentative kinetic interpretation was based on the expectation that the physiologic marrow response to removal of **neutrophils** from circulation would produce an increase in **promyelocytes** and **myelocytes** due to influx and proliferation, and a decrease in marrow segmented cells due to accelerated release. In two patients increased segmented marrow cells were consistent with an abnormality of release. Decreased numbers of **promyelocytes** and **myelocytes** in three patients was consistent with decreased proliferation. In five patients basal numbers of **promyelocytes** and **myelocytes** suggested abnormal proliferation or abnormal regulation of myelopoiesis. The number of **metamyelocytes** and band forms relative to **promyelocytes** and **myelocytes** was normal in all 10 patients: none had evidence of cell loss during postmitotic maturation. The term "chronic benign idiopathic neutropenia" appears to embrace more than one mechanism for neutropenia. All 10 patients had evidence of abnormal **neutrophil** marrow function.

TI **Neutrophil** marrow in chronic benign idiopathic neutropenia.

AB Quantitative studies of **neutrophil** marrow were carried out in 10 patients with chronic neutropenia (60 to 1,970 cells/microliter) with no other abnormalities and no serious infections. **Neutrophil** marrow cellularity was determined from **neutrophil**-normoblast ratios in marrow sections and ferrokinetic estimation of normoblasts. The results were interpreted in the **light** of 95 per cent confidence limits previously observed in 13 normal volunteer subjects. Three distinct **neutrophil** marrow profiles were determined by the numbers of **promyelocytes** and **myelocytes** and of segmented marrow cells. Tentative kinetic interpretation was based on the expectation that the physiologic marrow response to removal of **neutrophils** from circulation would produce an increase in **promyelocytes** and **myelocytes** due to influx and proliferation, and a decrease in marrow segmented cells due to accelerated release. In two patients increased segmented marrow cells were consistent with an abnormality of release. Decreased numbers of **promyelocytes** and **myelocytes** in three patients was consistent with decreased proliferation. In five patients basal numbers of **promyelocytes** and **myelocytes** suggested abnormal proliferation or abnormal regulation of myelopoiesis. The number of **metamyelocytes** and band forms relative to **promyelocytes** and **myelocytes** was normal in all 10 patients: none had evidence of cell loss during postmitotic maturation. The term "chronic benign idiopathic neutropenia" appears to embrace more than one mechanism for neutropenia. All 10 patients had evidence of abnormal **neutrophil** marrow function.

CT Check Tags: Female; Human; Male; Support, U.S. Gov't, Non-P.H.S.; Support,
U.S. Gov't, P.H.S.
Adult
Aged
*Agranulocytosis: PA, pathology
*Bone Marrow: PA, pathology
Bone Marrow: PP, physiopathology
Child, Preschool
Chronic Disease
Iron: BL, blood
Leukocyte Count
Middle Age
Neutropenia: BL, blood
*Neutropenia: PA, pathology
***Neutrophils: PA, pathology**

L102 ANSWER 9 OF 11 MEDLINE
81069968 Document Number: 81069968. Differential expression of lectin receptors during hemopoietic differentiation: enrichment for **granulocyte**-macrophage progenitor cells. Nicola N A; Burgess A W; Staber F G; Johnson G R; Metcalf D; Battye F L. JOURNAL OF CELLULAR PHYSIOLOGY, (1980 May) 103 (2) 217-37. Journal code: HNB. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB Molecular changes occur at the surface of hemopoietic cells during differentiation from progenitor cells to mature **granulocytes** and macrophages. The differential expression of surface carbohydrate residues has been probed using lectins and the results used to purify normal mouse **granulocyte**-macrophage progenitor cells. Ten different lectins were screened for selective interaction with mouse hemopoietic colony-forming cells (CFCs), using agglutination or a quantitative analysis of the number of fluoresceinated lectin molecules bound per cell using a fluorescence activated cell sorter (FACS). Pokeweed mitogen (PWM), Helix pomatia agglutinin (HPA), soybean agglutinin (SBA), and peanut agglutinin (PNA) preferentially bound to CFCs so that it was possible to enrich 4 to 10-fold for these progenitor cells by sorting for the highly fluorescent cells. Further analysis of the low and high angle **light scattering** characteristics of the CFCs indicated that these cells were polydisperse, but could be enriched ten-fold by selecting for cells with high intensity low angle (0 degrees) scatter and low intensity high angle (90 degrees) scatter. PWM gave the best enrichment (10 to 15-fold) for adult bone marrow CFCs, for CFCs from fetal sources (fetal liver, fetal blood), and for CFCs from the spleens of mice injected previously with outer membrane lipoprotein from E. coli. Three parameter sorting for CFC using the FACS (low angle scatter, high angle scatter, and PWM-fluorescence) resulted in large enrichment factors (16 to 50-fold) for CFCs from all the above sources. Over 7% of the cells sorted from bone marrow, 10% of the cells sorted from post-lipoprotein spleen, and 28% of the cells sorted from fetal peripheral blood were hemopoietic CFCs. Ninety percent of the cells in these fractions had the morphology of blast cells or **myelocytes**. Thus, it was possible to identify the morphological characteristics of the hemopoietic progenitor cells. Screening of other developmental systems using quantitation of fluorescence with lectins should prove of general value for the purification of selected differentiation states.

TI Differential expression of lectin receptors during hemopoietic differentiation: enrichment for **granulocyte**-macrophage progenitor cells.

AB Molecular changes occur at the surface of hemopoietic cells during differentiation from progenitor cells to mature **granulocytes** and macrophages. The differential expression of surface carbohydrate residues has been probed using lectins and the results used to purify normal mouse **granulocyte**-macrophage progenitor cells. Ten different lectins were screened for selective interaction with mouse hemopoietic colony-forming cells (CFCs), using agglutination or a quantitative analysis of the number of fluoresceinated lectin molecules bound per cell using a fluorescence activated cell sorter (FACS). Pokeweed mitogen (PWM), Helix pomatia agglutinin (HPA), soybean agglutinin (SBA), and peanut agglutinin (PNA) preferentially bound to CFCs so that it was possible to enrich 4 to 10-fold for these progenitor cells by sorting for the highly fluorescent cells. Further analysis of the low and high angle **light scattering** characteristics of the CFCs indicated

that these cells were polydisperse, but could be enriched ten-fold by selecting for cells with high intensity low angle (0 degrees) scatter and low intensity high angle (90 degrees) scatter. PWM gave the best enrichment (10 to 15-fold) for adult bone marrow CFCs, for CFCs from fetal sources (fetal liver, fetal blood), and for CFCs from the spleens of mice injected previously with outer membrane lipoprotein from E. coli. Three parameter sorting for CFC using the FACS (low angle scatter, high angle scatter, and PWM-fluorescence) resulted in large enrichment factors (16 to 50-fold) for CFCs from all the above sources. Over 7% of the cells sorted from bone marrow, 10% of the cells sorted from post-lipoprotein spleen, and 28% of the cells sorted from fetal peripheral blood were hemopoietic CFCs. Ninety percent of the cells in these fractions had the morphology of blast cells or **myelocytes**. Thus, it was possible to identify the morphological characteristics of the hemopoietic progenitor cells. Screening of other developmental systems using quantitation of fluorescence with lectins should prove of general value for the purification of selected differentiation states.

CT Check Tags: Animal; Male; Support, U.S. Gov't, P.H.S.
Cell Differentiation
Cells, Cultured
***Granulocytes: CY, cytology**
Granulocytes: ME, metabolism
*Hematopoietic Stem Cells: CY, cytology
Hematopoietic Stem Cells: ME, metabolism
Leukocyte Count
*Macrophages: CY, cytology
Macrophages: ME, metabolism
Mice
Mice, Inbred C57BL: PH, physiology
Mice, Inbred DBA: PH, physiology
*Receptors, Mitogen: ME, metabolism

L102 ANSWER 10 OF 11 MEDLINE

80109646 Document Number: 80109646. **Neutrophil** marrow profiles in patients with rheumatoid arthritis and neutropenia. Dancey J T; Brubaker L H. BRITISH JOURNAL OF HAEMATOLOGY, (1979 Dec) 43 (4) 607-17. Journal code: AXC. ISSN: 0007-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Neutrophil** marrow cellularity was determined in 14 neutropenic patients with rheumatoid arthritis (RA) from measurements of **neutrophil**-normoblast ratios in marrow biopsies and ferrokinetic estimates of marrow normoblasts. A marrow profile was developed for each patient comprising the numbers of **promyelocytes** and **myelocytes**, of **metamyelocytes** and bands, and of segmented **neutrophils** in whole marrow. In each case a maturation ratio was calculated by dividing the number of **metamyelocytes** and bands by the number of **promyelocytes** and **myelocytes**. The physiologic marrow response to loss of **neutrophils** from circulation was assumed to be an increase in **promyelocytes** and **myelocytes** due to proliferation and influx, a reduction in segmented cells due to early release, and a normal maturation ratio. The results were interpreted in the **light** of the 95% confidence limits for data previously obtained from 13 normal subjects: in patients with neutropenia reduced or basal numbers of **promyelocytes** and **myelocytes** were interpreted as absence of the anticipated proliferative response; increased numbers of marrow segmented cells were attributed to failure of release; a low maturation ratio was assessed to

reflect intramedullary cell loss. The pattern in two patients with Felty's syndrome was consistent with a physiological response to **neutrophil** destruction. The other 12 patients had **neutrophil** marrow abnormalities. Seven patients with Felty's syndrome and four patients without splenomegaly had absolute or relative hypoplasia of **neutrophil** marrow or low maturation ratios. One patient with a normal spleen size had an increased number of marrow segmented cells yet failed to mobilize cells normally in response to dialysis coil-activation of C3. Abnormalities of **neutrophil** marrow may contribute to neutropenia in RA irrespective of the presence of splenomegaly. Recognition of **neutrophil** marrow abnormalities in these patients may be of value in prognosis and management.

TI **Neutrophil** marrow profiles in patients with rheumatoid arthritis and neutropenia.

AB **Neutrophil** marrow cellularity was determined in 14 neutropenic patients with rheumatoid arthritis (RA) from measurements of **neutrophil**-normoblast ratios in marrow biopsies and ferrokinetic estimates of marrow normoblasts. A marrow profile was developed for each patient comprising the numbers of **promyelocytes** and **myelocytes**, of **metamyelocytes** and bands, and of segmented **neutrophils** in whole marrow. In each case a maturation ratio was calculated by dividing the number of **metamyelocytes** and bands by the number of **promyelocytes** and **myelocytes** . The physiologic marrow response to loss of **neutrophils** from circulation was assumed to be an increase in **promyelocytes** and **myelocytes** due to proliferation and influx, a reduction in segmented cells due to early release, and a normal maturation ratio. The results were interpreted in the **light** of the 95% confidence limits for data previously obtained from 13 normal subjects: in patients with neutropenia reduced or basal numbers of **promyelocytes** and **myelocytes** were interpreted as absence of the anticipated proliferative response; increased numbers of marrow segmented cells were attributed to failure of release; a low maturation ratio was assessed to reflect intramedullary cell loss. The pattern in two patients with Felty's syndrome was consistent with a physiological response to **neutrophil** destruction. The other 12 patients had **neutrophil** marrow abnormalities. Seven patients with Felty's syndrome and four patients without splenomegaly had absolute or relative hypoplasia of **neutrophil** marrow or low maturation ratios. One patient with a normal spleen size had an increased number of marrow segmented cells yet failed to mobilize cells normally in response to dialysis coil-activation of C3. Abnormalities of **neutrophil** marrow may contribute to neutropenia in RA irrespective of the presence of splenomegaly. Recognition of **neutrophil** marrow abnormalities in these patients may be of value in prognosis and management.

CT Check Tags: Female; Human; Male; Support, U.S. Gov't, P.H.S.
Adult
Aged
*Agranulocytosis: PA, pathology
Arthritis, Rheumatoid: BL, blood
Arthritis, Rheumatoid: CO, complications
*Arthritis, Rheumatoid: PA, pathology
*Bone Marrow: PA, pathology
Iron: BL, blood
Leukocyte Count
Middle Age
Mitosis

Neutropenia: BL, blood
Neutropenia: CO, complications
*Neutropenia: PA, pathology
***Neutrophils: PA, pathology**

L102 ANSWER 11 OF 11 MEDLINE DUPLICATE 1
77127140 Document Number: 77127140. The peripheral blood in chronic granulocytic leukaemia. Study of 50 untreated Philadelphia-positive cases.
Spiers A S; Bain B J; Turner J E. SCANDINAVIAN JOURNAL OF HAEMATOLOGY, (1977 Jan) 18 (1) 25-38. Journal code: UCV. ISSN: 0036-553X. Pub. country: Denmark. Language: English.
AB The haematological findings in the peripheral blood of 50 patients in whom the diagnosis of chronic granulocytic leukaemia (CGL) had been made in the haematology laboratory, and who were subsequently shown to be Philadelphia-chromosome-positive, have been reviewed. The differential **leucocyte counts** were performed by 3 observers, examining a total of 1,500 cells in each patient. The degree of anaemia at diagnosis was unrelated to sex and correlated poorly with **leucocyte count**; thrombocytopenia seemed unrelated to leucocytosis. A differential luecocyte count which included a complete spectrum of granulocytic cells, with prominent peaks in the percentages of **myelocytes** and **neutrophils**, was an invariable finding. Absolute **basophilia** occurred in all patients and absolute **eosinophilia** in 92%. In 54% of the patients there was an absolute lymphocytosis. Unlike the finding in normal subjects, there was no linear relationship between the numbers of circulating **neutrophils** and monocytes. Application of these findings should improve the accuracy of the haematological diagnosis of CGL, while study of the rare cases which possess the above features but are Ph1-negative may throw further **light** on the role of the Philadelphia chromosome in the natural history of CGL.
AB The haematological findings in the peripheral blood of 50 patients in whom the diagnosis of chronic granulocytic leukaemia (CGL) had been made in the haematology laboratory, and who were subsequently shown to be Philadelphia-chromosome-positive, have been reviewed. The differential **leucocyte counts** were performed by 3 observers, examining a total of 1,500 cells in each patient. The degree of anaemia at diagnosis was unrelated to sex and correlated poorly with **leucocyte count**; thrombocytopenia seemed unrelated to leucocytosis. A differential luecocyte count which included a complete spectrum of granulocytic cells, with prominent peaks in the percentages of **myelocytes** and **neutrophils**, was an invariable finding. Absolute **basophilia** occurred in all patients and absolute **eosinophilia** in 92%. In 54% of the patients there was an absolute lymphocytosis. Unlike the finding in normal subjects, there was no linear relationship between the numbers of circulating **neutrophils** and monocytes. Application of these findings should improve the accuracy of the haematological diagnosis of CGL, while study of the rare cases which possess the above features but are Ph1-negative may throw further **light** on the role of the Philadelphia chromosome in the natural history of CGL.
CT Check Tags: Female; Human; Male

Basophils
Blood Cell Count
Blood Platelets
*Chromosome Aberrations
*Chromosomes, Human, 21-22
Eosinophils
Hemoglobins: AN, analysis
*Leukemia, Myeloid: BL, blood
Leukemia, Myeloid: GE, genetics
Leukocyte Count
Lymphocytes
Monocytes
Neutrophils
Sex Factors

=> fil reg;s (fitc or fluorescein? isothiocyan? or phycoerythrin? or pe or allophicocyanin? or apc or texas red or pe cy5 or peridinin? chlorophyll protein or percp/cn)

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     2 FITC
   659 FLUORESCEIN?
 15687 ISOTHIOCYAN?
     8 FLUORESCEIN? ISOTHIOCYAN?
         (FLUORESCEIN?(W)ISOTHIOCYAN?)
    55 PHYCOERYTHRIN?
  1201 PE
   264 PES
  1462 PE
         (PE OR PES)
     0 ALLOPHICOCYANIN?
    94 APC
   123 TEXAS
  3809 RED
     5 TEXAS RED
         (TEXAS(W)RED)
  1201 PE
   264 PES
  1462 PE
         (PE OR PES)
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             1 CY5
             0 PE CY5
               (PE(W)CY5)
            31 PERIDININ?
           845 CHLOROPHYLL
             2 CHLOROPHYLLS
           845 CHLOROPHYLL
               (CHLOROPHYLL OR CHLOROPHYLLS)
        346446 PROTEIN
           493 PROTEINS
        346446 PROTEIN
               (PROTEIN OR PROTEINS)
             0 PERIDININ? CHLOROPHYLL PROTEIN
               (PERIDININ?(W)CHLOROPHYLL(W)PROTEIN)
             0 PERCP/CN
L103      1625 (FITC OR FLUORESCEIN? ISOTHIOCYAN? OR PHYCOERYTHRIN? OR PE OR
               ALLOPHICOCYANIN? OR APC OR TEXAS RED OR PE CY5 OR PERIDININ?
               CHLOROPHYLL PROTEIN OR PERCP/CN)

=> fil medl,caplus,biosis,embase,wpids,ntis,compendex,inspec

COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               54.30     247.31

FILE 'MEDLINE' ENTERED AT 12:46:48 ON 24 MAY 2000

FILE 'CAPLUS' ENTERED AT 12:46:48 ON 24 MAY 2000
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FILE 'INSPEC' ENTERED AT 12:46:48 ON 24 MAY 2000
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=> fil reg;s (fitc or fluorescein? isothiocyan? or phycoerythrin? or pe or
allophicocyanin? or apc or texas red or pe cy5 or peridinin? chlorophyll
protein or percp)/cn

COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                7.26     254.57
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FILE 'REGISTRY' ENTERED AT 12:47:05 ON 24 MAY 2000
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STRUCTURE FILE UPDATES: 23 MAY 2000 HIGHEST RN 266330-07-8
DICTIONARY FILE UPDATES: 23 MAY 2000 HIGHEST RN 266330-07-8

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 11, 2000

Please note that search-term pricing does apply when conducting SmartSELECT searches.

Structure search limits have been increased. See HELP SLIMIT for details.

'?' TRUNCATION SYMBOL NOT VALID WITHIN 'FLUORESCEIN? ISOTHIOCYAN?'
The truncation symbol ? may be used only at the end of a search term. To specify a variable character within a word use '!', e.g., 'wom!n' to search for both 'woman' and 'women'. Enter "HELP TRUNCATION" at an arrow prompt (=>) for more information.

=> s (fitc or fluorescein isothiocyanate or phycoerythrin or pe or allophicocyanin or apc or texas red or "pe-cy5" or peridinin chlorophyll protein or percp)/cn

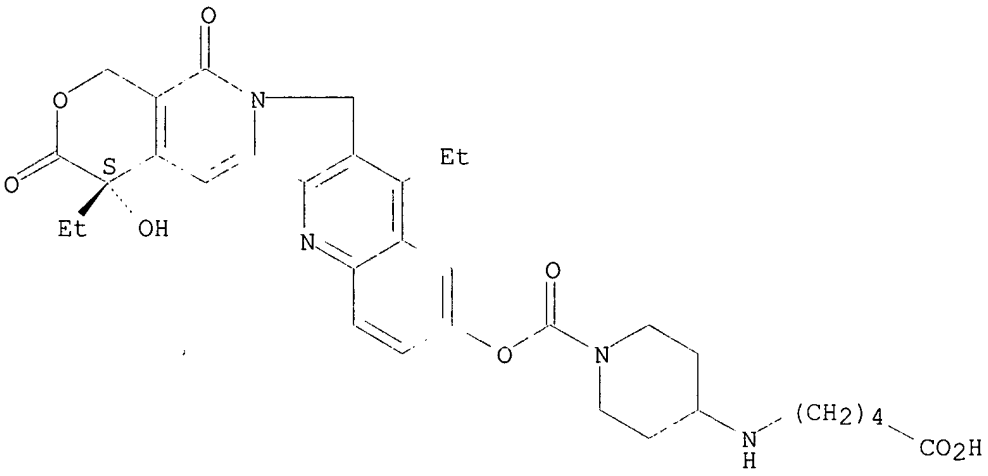
```
             1 FITC/CN
             1 FLUORESCEIN ISOTHIOCYANATE/CN
             0 PHYCOERYTHRIN/CN
             1 PE/CN
             0 ALLOPHICOCYANIN/CN
             6 APC/CN
             1 TEXAS RED/CN
             0 "PE-CY5"/CN
             0 PERIDININ CHLOROPHYLL PROTEIN/CN
             0 PERCP/CN
L104         9 (FITC OR FLUORESCEIN ISOTHIOCYANATE OR PHYCOERYTHRIN OR PE OR
               ALLOPHICOCYANIN OR APC OR TEXAS RED OR "PE-CY5" OR PERIDININ
               CHLOROPHYLL PROTEIN OR PERCP)/CN
```

=> d 1-9 ide can

L104 ANSWER 1 OF 9 REGISTRY COPYRIGHT 2000 ACS
RN 181467-56-1 REGISTRY
CN 1-Piperidinecarboxylic acid, 4-[(4-carboxybutyl)amino]-, 1-[(4S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl] ester (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 1-Piperidinecarboxylic acid, 4-[(4-carboxybutyl)amino]-, 1-(4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ester, (S)-
OTHER NAMES:
CN **APC**
CN RPR 121056
FS STEREOSEARCH
MF C33 H38 N4 O8
CI COM
SR CA

LC STN Files: CA, CAPLUS, TOXLIT, USPATFULL

Absolute stereochemistry.



8 REFERENCES IN FILE CA (1967 TO DATE)
8 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE 1: 132:245804

REFERENCE 2: 131:165300

REFERENCE 3: 131:39168

REFERENCE 4: 130:219737

REFERENCE 5: 130:177068

REFERENCE 6: 129:211171

REFERENCE 7: 129:197536

REFERENCE 8: 125:211705

L104 ANSWER 2 OF 9 REGISTRY COPYRIGHT 2000 ACS
RN 151438-87-8 REGISTRY
CN PE (catalyst promoter) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **PE**
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE 1: 119:271783

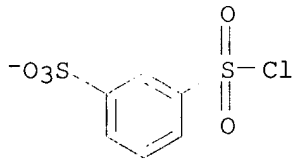
L104 ANSWER 3 OF 9 REGISTRY COPYRIGHT 2000 ACS

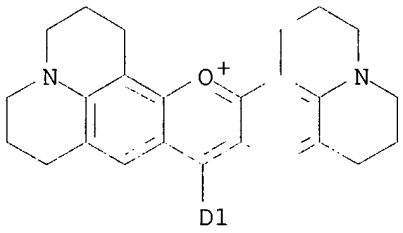
RN 101379-98-0 REGISTRY
CN APC (accelerator) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **APC**
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE 1: 104:154680

L104 ANSWER 4 OF 9 REGISTRY COPYRIGHT 2000 ACS
RN 82354-19-6 REGISTRY
CN 1H,5H,11H,15H-Xantheno[2,3,4-ij:5,6,7-i'j']diquinolizin-18-ium, 9-[2(or 4)-(chlorosulfonyl)-4(or 2)-sulfophenyl]-2,3,6,7,12,13,16,17-octahydro-, inner salt (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Sulforhodamine 101 Sulfonyl Chloride
CN **Texas Red**
MF C31 H29 Cl N2 O6 S2
CI IDS, COM
LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, CA, CANCERLIT, CAPLUS, CEN, CHEMCATS, CIN, CSCHEM, IFICDB, IFIUDB, MEDLINE, PROMT, TOXLINE, TOXLIT, USPATFULL





273 REFERENCES IN FILE CA (1967 TO DATE)
98 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
276 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE 1: 132:289573

REFERENCE 2: 132:289566

REFERENCE 3: 132:275152

REFERENCE 4: 132:262409

REFERENCE 5: 132:262405

REFERENCE 6: 132:262396

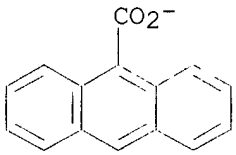
REFERENCE 7: 132:261369

REFERENCE 8: 132:246883

REFERENCE 9: 132:233995

REFERENCE 10: 132:205134

L104 ANSWER 5 OF 9 REGISTRY COPYRIGHT 2000 ACS
RN 81503-67-5 REGISTRY
CN 9-Anthracenecarboxylic acid, ion(1-) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 9-Anthracenecarboxylate
CN 9-Anthracenylcarboxylate
CN **ApC**
FS 3D CONCORD
MF C15 H9 O2
CI COM
LC STN Files: BEILSTEIN*, BIOSIS, CA, CAPLUS, GMELIN*, TOXLIT
(*File contains numerically searchable property data)



30 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
31 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE 1: 132:299563

REFERENCE 2: 132:207565

REFERENCE 3: 131:297859

REFERENCE 4: 131:240586

REFERENCE 5: 130:204265

REFERENCE 6: 128:41470

REFERENCE 7: 127:199793

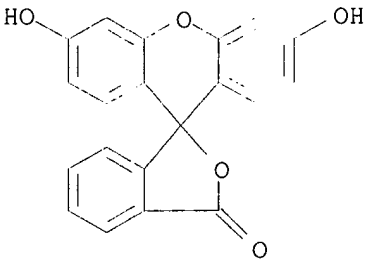
REFERENCE 8: 125:212254

REFERENCE 9: 125:30427

REFERENCE 10: 124:248665

L104 ANSWER 6 OF 9 REGISTRY COPYRIGHT 2000 ACS

RN 27072-45-3 REGISTRY
CN Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy-5(or 6)-isothiocyanato- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Fluorescein, isothiocyanato- (6CI, 8CI)
OTHER NAMES:
CN **FITC**
CN **Fluorescein isothiocyanate**
AR 25168-13-2
DR 64937-10-6, 28325-37-3, 29792-10-7
MF C21 H11 N O5 S
CI IDS, COM
LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAOLD, CAPLUS, CASREACT, CEN, CHEMCATS, CHEMLIST, CIN, CSCHEM, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, PIRA, PROMT, TOXLINE, TOXLIT, USPATFULL
Other Sources: EINECS**, NDSL**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)



1762 REFERENCES IN FILE CA (1967 TO DATE)
703 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1768 REFERENCES IN FILE CAPLUS (1967 TO DATE)
3 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 132:292723

REFERENCE 2: 132:289573

REFERENCE 3: 132:275152

REFERENCE 4: 132:262405

REFERENCE 5: 132:262397

REFERENCE 6: 132:262396

REFERENCE 7: 132:262248

REFERENCE 8: 132:262154

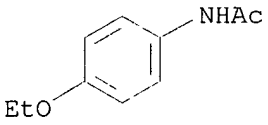
REFERENCE 9: 132:260666

REFERENCE 10: 132:249993

L104 ANSWER 7 OF 9 REGISTRY COPYRIGHT 2000 ACS
RN 8003-03-0 REGISTRY
CN Benzoic acid, 2-(acetyloxy)-, mixt. with 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione and N-(4-ethoxyphenyl)acetamide (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-, mixt. contg. (9CI)
CN Acetamide, N-(4-ethoxyphenyl)-, mixt. contg. (9CI)
OTHER NAMES:
CN Acetylsalicylic acid-caffeine-phenacetin mixt.
CN **APC**
CN APC (pharmaceutical)
CN Ascophen
CN Askophen
CN Aspirin, phenacetin and caffeine
CN Aspirin-caffeine-phenacetin mixt.
CN Citramon
CN Empirin compound
CN Kofitsil
CN Oscophen
CN P-A-C Compound
CN Thomapyrin
DR 8074-12-2, 37317-82-1, 91925-29-0, 52081-01-3
MF C10 H13 N O2 . C9 H8 O4 . C8 H10 N4 O2
CI MXS
LC STN Files: BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CHEMLIST, CSCHEM,
EMBASE, HSDB*, IMSDIRECTORY, PROMT, RTECS*, TOXLINE, TOXLIT
(*File contains numerically searchable property data)

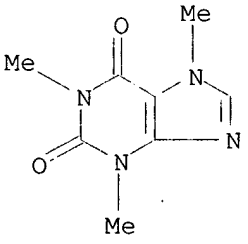
CM 1

CRN 62-44-2
CMF C10 H13 N O2



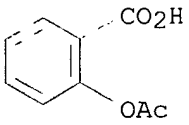
CM 2

CRN 58-08-2
CMF C8 H10 N4 O2



CM 3

CRN 50-78-2
CMF C9 H8 O4



46 REFERENCES IN FILE CA (1967 TO DATE)
46 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE 1: 130:158519

REFERENCE 2: 125:265924

REFERENCE 3: 125:184699

REFERENCE 4: 121:212837

REFERENCE 5: 121:18178

REFERENCE 6: 119:103437

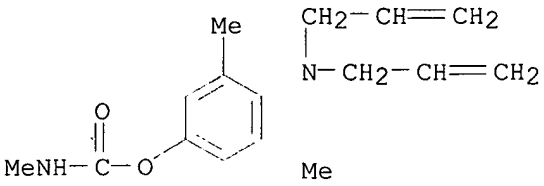
REFERENCE 7: 114:192680

REFERENCE 8: 112:204614

REFERENCE 9: 111:114434

REFERENCE 10: 111:84194

L104 ANSWER 8 OF 9 REGISTRY COPYRIGHT 2000 ACS
RN 6392-46-7 REGISTRY
CN Phenol, 4-(di-2-propenylamino)-3,5-dimethyl-, methylcarbamate (ester) (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 3,5-Xylenol, 4-(diallylamino)-, methylcarbamate (ester)
CN Carbamic acid, methyl-, 4-(diallylamino)-3,5-xylyl ester (7CI, 8CI)
OTHER NAMES:
CN 4-Diallylamino-3,5-dimethylphenyl methylcarbamate
CN 4-Diallylamino-3,5-dimethylphenyl N-methylcarbamate
CN 4-Diallylamino-3,5-xylyl methylcarbamate
CN 4-Diallylamino-3,5-xylyl N-methylcarbamate
CN Allyxycarb
CN **APC**
CN APC (pesticide)
CN BAY 50282
CN Hydrol
CN Hydrol (insecticide)
FS 3D CONCORD
MF C16 H22 N2 O2
CI COM
LC STN Files: AGRICOLA, BEILSTEIN*, BIOBUSINESS, BIOSIS, CA, CABA, CAOLD, CAPLUS, CHEMLIST, CSCHEM, MSDS-OHS, PIRA, PROMT, RTECS*, TOXLINE, TOXLIT, USPATFULL
(*File contains numerically searchable property data)
Other Sources: EINECS**
(**Enter CHEMLIST File for up-to-date regulatory information)



46 REFERENCES IN FILE CA (1967 TO DATE)
46 REFERENCES IN FILE CAPLUS (1967 TO DATE)
5 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 125:300474

REFERENCE 2: 123:93283

REFERENCE 3: 109:18504

REFERENCE 4: 108:208797

REFERENCE 5: 103:1655

REFERENCE 6: 101:146138

REFERENCE 7: 99:65537

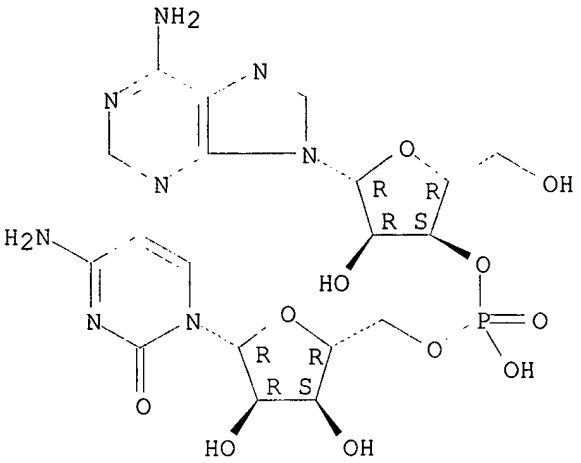
REFERENCE 8: 98:220404

REFERENCE 9: 97:90420

REFERENCE 10: 96:29650

L104 ANSWER 9 OF 9 REGISTRY COPYRIGHT 2000 ACS
RN 4833-63-0 REGISTRY
CN Cytidine, adenylyl-(3'.fwdarw.5')- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 3'-Adenylic acid, 5'-ester with cytidine (6CI)
CN Adenosine, cytidylyl-(5'.fwdarw.3')- (7CI, 8CI)
OTHER NAMES:
CN Adenylyl-(3'.fwdarw.5')-cytidine
CN Adenylylcytosine
CN **ApC**
CN GenBank C55081
FS NUCLEIC ACID SEQUENCE; STEREOSEARCH
DR 24016-30-6
MF C19 H25 N8 O11 P
CI COM
LC STN Files: BEILSTEIN*, BIOSIS, CA, CAOLD, CAPLUS, CASREACT, CHEMLIST, CSCHEM, GENBANK, MEDLINE, TOXLIT
(*File contains numerically searchable property data)
Other Sources: EINECS**
(**Enter CHEMLIST File for up-to-date regulatory information)

Absolute stereochemistry.



179 REFERENCES IN FILE CA (1967 TO DATE)
2 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
179 REFERENCES IN FILE CAPLUS (1967 TO DATE)
9 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 131:346488

REFERENCE 2: 131:99535

REFERENCE 3: 131:70281

REFERENCE 4: 130:22398

REFERENCE 5: 127:62375

REFERENCE 6: 125:320798

REFERENCE 7: 125:295393

REFERENCE 8: 124:81271

REFERENCE 9: 124:32143

REFERENCE 10: 123:249415

=> fil medl,caplus,biosis,embase,wpids,ntis,compendex,inspec

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	51.21	305.78

FILE 'MEDLINE' ENTERED AT 12:48:46 ON 24 MAY 2000

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=> s (fitc or fluorescein isothiocyanate or phycoerythrin or pe or
allophicocyanin or apc or texas red or "pe-cy5" or peridinin chlorophyll
protein or percp or l104

UNMATCHED LEFT PARENTHESIS '(FITC'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s (fitc or fluorescein? isothiocyan? or phycoerythrin? or pe or
allophicocyanin? or apc or texas red or "pe-cy5" or peridinin? chlorophyll
protein or percp or l104) and (l9 or l18)

L105 204 FILE MEDLINE
L106 12 FILE CAPLUS
L107 36 FILE BIOSIS
L108 147 FILE EMBASE
L109 1 FILE WPIDS
'CN' IS NOT A VALID FIELD CODE
L110 0 FILE NTIS
'CN' IS NOT A VALID FIELD CODE
L111 0 FILE COMPENDEX
'CN' IS NOT A VALID FIELD CODE
L112 0 FILE INSPEC

TOTAL FOR ALL FILES
L113 400 (FITC OR FLUORESCEIN? ISOTHIOCYAN? OR PHYCOERYTHRIN? OR PE OR
ALLOPHICOCYANIN? OR APC OR TEXAS RED OR "PE-CY5" OR PERIDININ?
CHLOROPHYLL PROTEIN OR PERCP OR L104) AND (L9 OR L18)

=> s l113 and (flow cytome? or flow cytometry/ct or cytomet? or
e5.196.172.382.240.350/ct or e5.909.262.386.350/ct)

L114 70 FILE MEDLINE
L115 6 FILE CAPLUS
L116 15 FILE BIOSIS
L117 33 FILE EMBASE
L118 1 FILE WPIDS
L119 0 FILE NTIS
L120 0 FILE COMPENDEX
L121 0 FILE INSPEC

TOTAL FOR ALL FILES
L122 125 L113 AND (FLOW CYTOME? OR FLOW CYTOMETRY/CT OR CYTOMET? OR
E5.19

6.172.382.240.350/CT OR E5.909.262.386.350/CT)

=> s (light(3a)scatter? or light scatter/ct or turidimet?) and l122

L123 11 FILE MEDLINE
L124 0 FILE CAPLUS
L125 5 FILE BIOSIS
L126 9 FILE EMBASE
L127 0 FILE WPIDS
L128 0 FILE NTIS
L129 0 FILE COMPENDEX
L130 0 FILE INSPEC

TOTAL FOR ALL FILES
L131 25 (LIGHT(3A) SCATTER? OR LIGHT SCATTER/CT OR TURIDIMET?) AND L122

=> s l131 not (173 or l36 or l101)

L132 11 FILE MEDLINE
L133 0 FILE CAPLUS
L134 5 FILE BIOSIS
L135 9 FILE EMBASE
L136 0 FILE WPIDS
L137 0 FILE NTIS
L138 0 FILE COMPENDEX
L139 0 FILE INSPEC

TOTAL FOR ALL FILES
L140 25 L131 NOT (L73 OR L36 OR L101)

=> dup rem l140

PROCESSING COMPLETED FOR L140
L141 14 DUP REM L140 (11 DUPLICATES REMOVED)

=> d cbib abs hit 1-14

L141 ANSWER 1 OF 14 MEDLINE DUPLICATE 1
1998239205 Document Number: 98239205. Single platform **flow** **cytometric** absolute CD34+ cell counts based on the ISHAGE guidelines. International Society of Hematotherapy and Graft Engineering [see comments]. Keeney M; Chin-Yee I; Weir K; Popma J; Nayar R; Sutherland D R. (The London Health Sciences Centre, Ontario, Canada.) CYTOMETRY, (1998 Apr 15) 34 (2) 61-70. Journal code: D92. ISSN: 0196-4763. Pub. country: United States. Language: English.

AB In concert with the International Society of Hematotherapy and Graft Engineering (ISHAGE), we previously described a set of guidelines for detection of CD34+ cells based on a four-parameter **flow** **cytometry** method (CD45 **FITC**/CD34 **PE** staining, side and forward angle **light scatter**). With this procedure, an absolute CD34+ count is generated by incorporating the **leukocyte count** from an automated hematology analyser (two-platform method). In the present study, we modified the basic ISHAGE method with the addition of a known number of Flow-Count fluorospheres. To reduce errors inherent to sample washing/centrifugation, we implemented ammonium chloride lyse, no-wash no-fix sample processing. These modifications convert the basic protocol into a single-platform method to determine the absolute CD34 count directly from a **flow**

cytometer and form the basis of the Stem-Kit from
Coulter/Immunotech. A total of 72 samples of peripheral blood, apheresis
packs, and cord blood were analysed and compared using the ISHAGE
protocol
with or without the addition of fluorescent microspheres. Comparison of
methods showed a high correlation coefficient (r=0.99), with no
statistically significant difference or bias between methods (P > 0.05).
Linearity of the absolute counting method generated an R2 value of 1.00
over the range of 0-250/microl. Precision of the absolute counting method
measured at three concentrations of CD34+-stabilised KG1 a cells
(Stem-Trol, COULTER) generated a coefficient of variation (C.V.) ranging
from 4% to 9.9%. In a further modification of the single-platform method,
the viability dye 7-amino actinomycin D was included and demonstrated
that
both viable and nonviable CD34+ cells could be identified and
quantitated.
Together, these modifications combine the accuracy and sensitivity of the
original ISHAGE method with the ability to produce an absolute count of
viable CD34+ cells. It is the accurate determination of this value that
is
most clinically relevant in the transplant setting. These modifications
may improve the interlaboratory reproducibility of CD34 determinations
due
to the reduction in sample handling and calculation of results.
TI Single platform **flow cytometric** absolute CD34+ cell
counts based on the ISHAGE guidelines. International Society of
Hematotherapy and Graft Engineering [see comments].
AB In concert with the International Society of Hematotherapy and Graft
Engineering (ISHAGE), we previously described a set of guidelines for
detection of CD34+ cells based on a four-parameter **flow**
cytometry method (CD45 **FITC**/CD34 **PE** staining,
side and forward angle **light scatter**). With this
procedure, an absolute CD34+ count is generated by incorporating the
leukocyte count from an automated hematology analyser
(two-platform method). In the present study, we modified the basic ISHAGE
method with the addition of a known number of Flow-Count fluorospheres.
To
reduce errors inherent to sample washing/centrifugation, we implemented
ammonium chloride lyse, no-wash no-fix sample processing. These
modifications convert the basic protocol into a single-platform method to
determine the absolute CD34 count directly from a **flow**
cytometer and form the basis of the Stem-Kit from
Coulter/Immunotech. A total of 72 samples of peripheral blood, apheresis
packs, and cord blood were analysed and compared using the ISHAGE
protocol
with or without the addition of fluorescent microspheres. Comparison of
methods showed a high correlation coefficient (r=0.99), with no
statistically significant difference or bias between methods (P > 0.05).
Linearity of the absolute counting method generated an R2 value of 1.00
over the range of 0-250/microl. Precision of the absolute counting method
measured at three concentrations of CD34+-stabilised KG1 a cells
(Stem-Trol, COULTER) generated a coefficient of variation (C.V.) ranging
from 4% to 9.9%. In a further modification of the single-platform method,
the viability dye 7-amino actinomycin D was included and demonstrated
that
both viable and nonviable CD34+ cells could be identified and
quantitated.
Together, these modifications combine the accuracy and sensitivity of the
original ISHAGE method with the ability to produce an absolute count of
viable CD34+ cells. It is the accurate determination of this value that
is

most clinically relevant in the transplant setting. These modifications may improve the interlaboratory reproducibility of CD34 determinations due to the reduction in sample handling and calculation of results.

CT Check Tags: Human
*Antigens, CD34: AN, analysis
Cell Survival
***Flow Cytometry: MT, methods**
Hematopoietic Stem Cells: CY, cytology
Hematopoietic Stem Cells: IM, immunology
***Leukocyte Count**
Leukocytes, Mononuclear: CY, cytology
Leukocytes, Mononuclear: IM, immunology
Linear Models
Reproducibility of Results

L141 ANSWER 2 OF 14 MEDLINE DUPLICATE 2

1998071606 Document Number: 98071606. Demonstration by **flow cytometry** of the numbers of residual white blood cells and platelets in filtered red blood cell concentrates and plasma preparations.

Neumuller J; Schwartz D W; Mayr W R. (Ludwig Boltzmann Institute for Rheumatology and Balneology, Vienna, Austria.) VOX SANGUINIS, (1997) 73 (4) 220-9. Journal code: XLI. ISSN: 0042-9007. Pub. country: Switzerland.

Language: English.

AB BACKGROUND AND OBJECTIVES: New-generation polyester filters provide significant depletion of white blood cells (WBC) and platelets (PLT) in filtered red blood cell concentrates (FRCC) and in filtered plasma preparations (FP). The aim of this study was to elaborate a sensitive **flow cytometric** method for monitoring residual WBC and PLT in FRCC and FP. MATERIALS AND METHODS: We determined the number of WBC in 500 microliters FRCC of FP using 50 microliters of a combination of monoclonal antibodies (MAB) against CD45 (**FITC** labeled) and CD19 (**PE** labeled). After lysis of red blood cells, we mixed a specific number of reference beads with the remaining WBC. The number of residual WBC related to the acquisition volume was defined by the acquired reference beads. Using this method, the detection limit (DL) was 3 WBC/microliter. Alternative methods used MAB against CD45 (**FITC** and **PerCP** labeled) and CD14 (**PE** labeled) or lymphocyte subsets such as CD3 (**FITC** labeled) and CD19, CD4, CD8, CD16 and CD56 (**PE** labeled) in combination with CD45 (**PerCP** labeled). The DL values were 10 WBC/microliter for the CD45/CD14 staining and 0.1 WBC/microliter for the determination of both CD3+ and CD19+ lymphocytes. For residual PLT in FRCC or FP, we used an **FITC** -conjugated MAB against CD41, with reference beads to determine the acquisition volume. PLT were demonstrated in a green-fluorescence (FL1) single histogram after gating in the forward **light scatter** x 90 degrees **light scatter** signal dot plot. PLT counting was as described for WBC. The DL value was about 2 PLT/microliter. RESULTS: Filtration with Pall WBF-1 filters reduces WBC by 4 log and PLT by 3-4 log, resulting in cell counts which are below the critical limit for causing adverse transfusion reactions. CONCLUSIONS: **Flow cytometry** techniques provide a reproducible and objective tool for counting residual WBC and PLT in blood preparations compared with the Nageotte hemocytometer. Absolute numbers of leukocyte and lymphocyte subpopulations are obtainable.

TI Demonstration by **flow cytometry** of the numbers of

residual white blood cells and platelets in filtered red blood cell concentrates and plasma preparations.

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CT Check Tags: Human
Antibodies, Monoclonal
Antigens, CD14: IM, immunology
Antigens, CD45: IM, immunology
*Erythrocytes: CY, cytology
Filtration
***Flow Cytometry**
Fluorescein-5-isothiocyanate
***Leukocyte Count: MT, methods**
Microspheres
*Plasma: CY, cytology
*Platelet Count: MT, methods
Reproducibility of Results

L141 ANSWER 3 OF 14 MEDLINE DUPLICATE 3

97356399 Document Number: 97356399. How should CD34+ cells be analysed? A study of three classes of antibody and five leucocyte preparation procedures. Macey M G; McCarthy D A; van Agthoven A; Newland A C. (Department of Haematology, Royal London Hospital, Whitechapel, UK.) JOURNAL OF IMMUNOLOGICAL METHODS, (1997 May 26) 204 (2) 175-88. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB For patients undergoing stem cell transplantation after intensive marrow ablative therapy it is important to enumerate the CD34+ stem cells in peripheral blood so that the harvest can be timed in order to maximize the

number of cells collected by leucophoresis for subsequent haematopoietic reconstitution. The use of rapid **flow cytometric** techniques for the determination CD34+ **leucocyte numbers** has been advocated, although there is no consensus as to the best method. In this study, we have examined the effects of preparation procedures for **flow cytometry** on the binding of four CD34 antibodies (Immu-133, QBEND-10, HPCA2 and BIRMA-K3) to the three classes of epitopes on leucocytes. Whole blood, bone marrow and leucophoresis samples were analysed either directly after labelling with a vital nuclear dye (LDS-751) and fluorochrome-conjugated antibodies or after additional erythrocyte lysis and leucocyte fixation using four commercially available reagents (Q-Prep, OptiLyse B, OptiLyse C and FACS Lysing Solution). By comparison with the results obtained from viable leucocytes in unmanipulated samples, it was found that the binding of all four antibodies could be affected by lysis and fixation procedures and that the binding of the class I antibody Immu-133 was most markedly decreased. We conclude that CD34+ cells are best analysed using a whole blood procedure in which nucleated cells are identified by their side **light scatter** and the fluorescence associated with a vital nuclear dye (in this instance LDS-751) and the CD34+ cells are detected with **fluorescein isothiocyanate**- or **phycoerythrin** -conjugated antibodies.

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CT Check Tags: Animal; Human
Antibodies: IM, immunology
*Antigens, CD34: AN, analysis
Antigens, CD45: AN, analysis
Flow Cytometry
Hematopoietic Stem Cell Transplantation
*Hematopoietic Stem Cells
Leukapheresis
Leukemia: BL, blood

*Leukemia: TH, therapy
Leukocyte Count
Mice
Reticulocytes: IM, immunology

L141 ANSWER 4 OF 14 MEDLINE DUPLICATE 4
97442285 Document Number: 97442285. Analysis of variation in results of **flow cytometric** lymphocyte immunophenotyping in a multicenter study. Gratama J W; Kraan J; Van den Beemd R; Hooibrink B; Van Bockstaele D R; Hooijkaas H. (Department of Clinical and Tumor Immunology, Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.. gratama@immh.azr.nl) . CYTOMETRY, (1997 Aug 15) 30 (4) 166-77. Journal code: D92. ISSN: 0196-4763. Pub. country: United States. Language: English.

AB Fifty-five laboratories participated in a send-out study of four peripheral blood samples comparing a standard protocol vs. local protocols for **flow cytometric** lymphocyte immunophenotyping. The standard protocol included centrally provided reagents, instrument setup using triple-fluorescent microbeads and a three-color, whole-blood immunostaining technique based on **fluorescein isothiocyanate** and **phycoerythrin**-labeled monoclonal antibodies, erythrocyte lysis, washing, fixation, and identification of nucleated cells by the DNA/RNA stain LDS-751. Data analysis guidelines included lymphocyte selection using CD45,CD14-assisted "backgating" on forward (FSC) and sideward (SSC) **light scatter** and placement of fluorescence (FL) markers on the basis of the isotype control staining. Most (i.e., 77%) of the variation in results of percentage lymphocyte subset assessments using the standard protocol was explained by laboratory, sample, background FL, and the interaction between laboratory and sample. Purity and completeness of the FSC,SSC lymphogate, background FL, **flow cytometer** type, and **flow cytometer** setup (which were either partly or entirely determined by laboratory) contributed significantly to the variation. The effect of the **leukocyte** differential **count** on the variation in absolute numbers of lymphocyte subsets was particularly large in lymphopenic samples. The use of this standard protocol vs. local protocols did not reduce the interlaboratory variation. Instrument incompatibility with the standard protocol (e.g., incompatible filter combinations for LDS-751 detection) and lack of experience of many participants with three-color **flow cytometry** (in particular with the use of LDS-751) may have contributed to that result. We suggest that training and experience in a universally applicable standard protocol are critical for minimization of interlaboratory variation in **flow cytometric** immunophenotyping.

TI Analysis of variation in results of **flow cytometric** lymphocyte immunophenotyping in a multicenter study.

AB Fifty-five laboratories participated in a send-out study of four peripheral blood samples comparing a standard protocol vs. local protocols for **flow cytometric** lymphocyte immunophenotyping. The standard protocol included centrally provided reagents, instrument setup using triple-fluorescent microbeads and a three-color, whole-blood immunostaining technique based on **fluorescein isothiocyanate** and **phycoerythrin**-labeled monoclonal antibodies, erythrocyte lysis, washing, fixation, and identification of

nucleated cells by the DNA/RNA stain LDS-751. Data analysis guidelines included lymphocyte selection using CD45,CD14-assisted "backgating" on forward (FSC) and sideward (SSC) **light scatter** and placement of fluorescence (FL) markers on the basis of the isotype control staining. Most (i.e., 77%) of the variation in results of percentage lymphocyte subset assessments using the standard protocol was explained by laboratory, sample, background FL, and the interaction between laboratory and sample. Purity and completeness of the FSC,SSC lymphogate, background FL, **flow cytometer** type, and **flow cytometer** setup (which were either partly or entirely determined by laboratory) contributed significantly to the variation. The effect of the **leukocyte** differential **count** on the variation in absolute numbers of lymphocyte subsets was particularly large in lymphopenic samples. The use of this standard protocol vs. local protocols did not reduce the interlaboratory variation. Instrument incompatibility with the standard protocol (e.g., incompatible filter combinations for LDS-751 detection) and lack of experience of many participants with three-color **flow cytometry** (in particular with the use of LDS-751) may have contributed to that result. We suggest that training and experience in a universally applicable standard protocol are critical for minimization of interlaboratory variation in **flow cytometric** immunophenotyping.

CT Check Tags: Comparative Study; Human; Support, Non-U.S. Gov't
Antibodies, Monoclonal
*Antigens, CD: AN, analysis
Europe
***Flow Cytometry: MT, methods**
Flow Cytometry: ST, standards
Fluorescein-5-isothiocyanate
*Immunophenotyping: MT, methods
Immunophenotyping: ST, standards
Laboratories: ST, standards
Lymphocyte Subsets: IM, immunology
Lymphopenia: DI, diagnosis
Lymphopenia: IM, immunology
Phycoerythrin
Quality Control
Reproducibility of Results

RN **11016-17-4 (Phycoerythrin)**; 3326-32-7 (Fluorescein-5-isothiocyanate)

L141 ANSWER 5 OF 14 MEDLINE

95123121 Document Number: 95123121. Multicolor **flow cytometric** analysis of the CD45 antigen provides improved lymphoid cell discrimination in bone marrow and tissue biopsies. Festin R; Bjorkland A; Totterman T H. (Section for Clinical Immunology and Transfusion Medicine, University Hospital, Uppsala, Sweden..) JOURNAL OF IMMUNOLOGICAL METHODS, (1994 Dec 28) 177 (1-2) 215-24. Journal code: IFE.

ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Samples from bone marrow or non-hematopoietic tissue such as solid organ biopsies often contain an excess of non-leukocytes exhibiting lymphocyte-like **light scatter** characteristics, making it sometimes difficult to define satisfactory **light scatter** lymphocyte gates. To circumvent this, we describe here a multiparametric method of identifying lymphoid cells by expression of the CD45 antigen, in conjunction with **light scatter** parameters. A 'third color'-conjugated anti-CD45 antibody was included

with every **FITC/PE** double staining, thereby permitting
live or list mode analysis gating on CD45 positive cells. The
triple-staining technique was applied to (a) human bone marrow, showing
that special attention has to be given to the enumeration of B cells, and
(b) to liver biopsies, where gating on CD45 fluorescence and orthogonal
light scatter was shown to clearly resolve all
lymphocyte subsets from debris. All cell types examined in tissue
biopsies
as well as T and NK cells in bone marrow were best distinguished by
gating
on bright CD45 expression in conjunction with low orthogonal **light
scatter**, while accurate identification of marrow B cells relied
upon including all levels of CD45 intensity. The multicolor gating
procedure, aimed mainly at immune-monitoring of non-malignant tissues, is
applicable to most kinds of single cell samples, and may prove to be an
aid for lymphocyte gating in cases where leukocyte populations are not
clearly resolved on a **light scatter** basis alone.

TI Multicolor **flow cytometric** analysis of the CD45
antigen provides improved lymphoid cell discrimination in bone marrow and
tissue biopsies.

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biopsies often contain an excess of non-leukocytes exhibiting
lymphocyte-like **light scatter** characteristics, making
it sometimes difficult to define satisfactory **light
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multiparametric method of identifying lymphoid cells by expression of the
CD45 antigen, in conjunction with **light scatter**
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applicable to most kinds of single cell samples, and may prove to be an
aid for lymphocyte gating in cases where leukocyte populations are not
clearly resolved on a **light scatter** basis alone.

CT Check Tags: Human
Antibodies, Monoclonal: DU, diagnostic use
Antigens, CD: AN, analysis
*Antigens, CD45: AN, analysis
*Bone Marrow: CY, cytology
Carotenoids: DU, diagnostic use
***Flow Cytometry: MT, methods**
Lymphocyte Count
*Lymphocytes: CY, cytology
Protozoan Proteins: DU, diagnostic use

L141 ANSWER 6 OF 14 MEDLINE DUPLICATE 5
95336545 Document Number: 95336545. **Flow cytometric**
three-color determination of CD4 T-lymphocytes on blood specimens from
AIDS patients who have a large number of contaminating non-lymphocytes.
Pattanapanyasat K; Pengruangrojanachai V; Thepthai C; Suwanagool S; Wasi

C. (Thalassemia Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.) ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY, (1994 Dec) 12 (2) 105-9. Journal code: ABB. ISSN: 0125-877X. Pub. country: Thailand. Language: English.

AB A three-color **flow cytometric** determination of CD4 T-lymphocytes on whole blood specimens from AIDS patients which contain a high proportion of non-lymphocyte elements is described. Peripheral blood cells were stained by a three-color method using monoclonal antibodies conjugated respectively with **fluorescein isothiocyanate** (**FITC**)-CD3, **phycoerythrin** (**PE**)-CD4 and **peridinin chlorophyll protein** (**PerCP**)-CD45. CD45 stains all leukocytes with the highest fluorescence expression of CD45 antigen in lymphocytes. By combining **light scatter** with CD45 in the fluorescence 3 (FL3) channel, a **light scattering** window can be drawn to include almost all bright CD45 lymphocytes. This live gate of lymphocytes was then acquired and analysed simultaneously using other irrelevant two-color (**FITC/PE**) antibodies of CD3 and CD4 in the **FITC** and **PE** channels, respectively. This method is easy and straightforward, and gives successful analysis of CD4 T-lymphocytes in AIDS blood specimens contaminated with an unusually large number of non-lymphocytic cells.

TI **Flow cytometric** three-color determination of CD4 T-lymphocytes on blood specimens from AIDS patients who have a large number of contaminating non-lymphocytes.

AB A three-color **flow cytometric** determination of CD4 T-lymphocytes on whole blood specimens from AIDS patients which contain a high proportion of non-lymphocyte elements is described. Peripheral blood cells were stained by a three-color method using monoclonal antibodies conjugated respectively with **fluorescein isothiocyanate** (**FITC**)-CD3, **phycoerythrin** (**PE**)-CD4 and **peridinin chlorophyll protein** (**PerCP**)-CD45. CD45 stains all leukocytes with the highest fluorescence expression of CD45 antigen in lymphocytes. By combining **light scatter** with CD45 in the fluorescence 3 (FL3) channel, a **light scattering** window can be drawn to include almost all bright CD45 lymphocytes. This live gate of lymphocytes was then acquired and analysed simultaneously using other irrelevant two-color (**FITC/PE**) antibodies of CD3 and CD4 in the **FITC** and **PE** channels, respectively. This method is easy and straightforward, and gives successful analysis of CD4 T-lymphocytes in AIDS blood specimens contaminated with an unusually large number of non-lymphocytic cells.

CT Check Tags: Human; Male; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

*Acquired Immunodeficiency Syndrome: BL, blood
Acquired Immunodeficiency Syndrome: IM, immunology
Antibodies, Monoclonal
Antigens, CD3: AN, analysis
Antigens, CD4: AN, analysis
Antigens, CD45: AN, analysis
CD4 Lymphocyte Count: MT, methods
*CD4-Positive T-Lymphocytes: IM, immunology
***Flow Cytometry: MT, methods**
Fluorescent Dyes
*HIV Seropositivity: BL, blood
HIV Seropositivity: IM, immunology
Immunophenotyping

L141 ANSWER 7 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
94294921 EMBASE Document No.: 1994294921. Automated enumeration of CD34+

cells in peripheral blood and bone marrow. Chia Huei Chen; Lin W.; Shye S.; Kibler R.; Grenier K.; Recktenwald D.; Terstappen L.W.M.M.. B. Dickinson Immunocytometry Systems, 2350 Qume Drive,San Jose, CA 95131, United States. Journal of Hematotherapy 3/1 (3-13) 1994. ISSN: 1061-6128. CODEN: JOEMEL. Pub. Country: United States. Language: English. Summary Language: English.

AB We have developed a rapid and accurate method to enumerate the number of CD34+ cells in peripheral blood, bone marrow, and leukopheresis samples. The method consists of a two-tube assay and a dedicated software program for data acquisition and analysis. The first reagent combination consists of (a) a nucleic acid dye to identify nucleated cells, (b) a CD45 monoclonal antibody labeled with **PE/CY5** to discriminate progenitor cells from mature lymphoid, neutrophil, erythroid, and monocytic cells, (c) an IgG1 control antibody labeled with **PE** to establish the boundary between specific and nonspecific staining, and (d) a known number of fluorescent beads to determine an absolute count of cells. In the second reagent combination the IgG1 control antibody is replaced by a CD34 antibody labeled with **PE** that is used to identify the CD34+ cells in the location established by the control reagent combination. The software program uses the fluorescent beads to adjust the forward **light scatter**, orthogonal **light scatter**, and three fluorescence detectors of the **flow cytometer**. The expected location of the CD34+ cells is then established with the control reagent combination followed by the enumeration of the CD34+ cells per microliter of sample with the reagent combination containing the CD34 antibody. This method is sensitive enough to detect CD34+ cells in peripheral blood of normal donors and can reliably determine an increase in CD34+ cells in the peripheral blood of patients treated with chemotherapy and/or growth factors. The method alleviates some of the difficulties encountered when small numbers of CD34+ cells are enumerated. The system allows for more precise evaluations of the grafts used for bone marrow transplantation.

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of the grafts used for bone marrow transplantation.
CT Medical Descriptors:
*bone marrow transplantation
*leukapheresis
*leukemia: TH, therapy
article
autoanalysis
cell subpopulation
clinical article
computer program
controlled study
flow cytometry
fluorescence activated cell sorter
human
human cell
immunocytochemistry
leukocyte count
priority journal
Drug Descriptors:
*cd34 antigen: EC, endogenous compound
immunoglobulin g antibody
monoclonal antibody cd45: DV, drug development
unclassified drug

L141 ANSWER 8 OF 14 MEDLINE
93359721 Document Number: 93359721. A simple **flow cytometric** procedure for the determination of surface antigens on unfixed leucocytes in whole blood. McCarthy D A; Macey M G. (School of Biological Sciences, Queen Mary and Westfield College, London, UK..) JOURNAL OF IMMUNOLOGICAL METHODS, (1993 Aug 9) 163 (2) 155-60. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB A novel procedure has been developed for the quantitation by **flow cytometry** of function-associated antigens on neutrophils and monocytes in unlysed, unfixed, peripheral blood samples. Freshly drawn blood anticoagulated with the serine esterase inhibitor, phenylmethylsulphonyl fluoride, is mixed with the vital nucleic acid stain, LDS-751, labelled with monoclonal antibodies for 5 min at 4 degrees C, diluted and analysed in a five-parameter **flow cytometer**. The three major leucocyte subpopulations (neutrophils, lymphocytes and monocytes) can be resolved in real time on the basis of their side **light scattering** and staining intensity with LDS-751 in the FL3 channel (erythrocytes and platelets stain very weakly), whilst the fluorescence intensity due to bound **fluorescein isothiocyanate**- or **phycoerythrin** -labelled antibody is monitored simultaneously in the FL1 or FL2 channels respectively. This procedure avoids potential artefacts that can occur due to the use of fixatives, erythrocyte lysing agents, or anticoagulants which are also divalent metal ion chelators. It should be widely applicable for the quantitation of those function-associated antigens, such as adhesion molecules and immune complex receptors, whose surface expression can be rapidly upregulated following activation, as well as for the quantitation of those leucocyte surface antigens whose expression is not subject to rapid modulation.
TI A simple **flow cytometric** procedure for the determination of surface antigens on unfixed leucocytes in whole blood.
AB A novel procedure has been developed for the quantitation by **flow cytometry** of function-associated antigens on neutrophils and monocytes in unlysed, unfixed, peripheral blood samples. Freshly drawn

blood anticoagulated with the serine esterase inhibitor, phenylmethylsulphonyl fluoride, is mixed with the vital nucleic acid stain, LDS-751, labelled with monoclonal antibodies for 5 min at 4 degrees C, diluted and analysed in a five-parameter **flow cytometer**. The three major leucocyte subpopulations (neutrophils, lymphocytes and monocytes) can be resolved in real time on the basis of their side **light scattering** and staining intensity with LDS-751 in the FL3 channel (erythrocytes and platelets stain very weakly), whilst the fluorescence intensity due to bound **fluorescein isothiocyanate**- or **phycoerythrin** -labelled antibody is monitored simultaneously in the FL1 or FL2 channels respectively. This procedure avoids potential artefacts that can occur due to the use of fixatives, erythrocyte lysing agents, or anticoagulants which are also divalent metal ion chelators. It should be widely applicable for the quantitation of those function-associated antigens, such as adhesion molecules and immune complex receptors, whose surface expression can be rapidly upregulated following activation, as well as for the quantitation of those leucocyte surface antigens whose expression is not subject to rapid modulation.

CT Check Tags: Human
*Antigens, Surface: AN, analysis
***Flow Cytometry: MT, methods**
Fluorescent Dyes
Leukocyte Count
*Leukocytes: IM, immunology
Lymphocytes: IM, immunology
Monocytes: IM, immunology
Neutrophils: IM, immunology
Phenylmethylsulfonyl Fluoride
Staining: MT, methods

L141 ANSWER 9 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
92265347 EMBASE Document No.: 1992265347. Bone marrow cell differential counts obtained by multidimensional **flow cytometry**.
Terstappen L.W.M.M.; Levin J.. Becton Dickinson Immunocytom. Syst., 2350 Qume Drive,San Jose, CA 95131, United States. Blood Cells 18/2 (311-330) 1992.
ISSN: 0340-4684. CODEN: BLCEDD. Pub. Country: United States. Language: English. Summary Language: English.

AB Five-dimensional **flow cytometric** analysis of normal bone marrow aspirates was utilized to determine the frequency of neutrophils, eosinophils, monocytes, lymphocytes, nucleated erythrocytes, reticulocytes, platelets, and a cell population that included blasts of each of the cell lineages, megakaryocytes, plasma cells, and basophils. Each of these bone marrow cell populations had unique features with respect to forward **light scatter**, orthogonal **light scatter**, and staining with Thiazole-Orange, LDS-751, and CD45 labeled with **Phycoerythrin** (**PE**). The identity of the cell populations was verified by sorting each of the cell populations and subsequent light microscopic examination of the cells. The frequencies of the nucleated bone marrow cell subpopulations of 50 normal donors were for neutrophils, mean 72.3%; SD .+-. 5.1; 95% limits, 70.9-73.8%; eosinophils, mean 1.8%; SD .+-. 1.3; 95% limits, 1.4-2.1%; monocytes, mean, 2.8%; SD .+-. 1.2; 95% limits, 2.5-3.1%; lymphocytes, mean 12.1%; SD .+-. 3.6; 95% limits 11.1-13.2%; nucleated erythrocytes, mean 8.9%; SD .+-. 3.9; 95% limits, 7.8-10.1%; and the cell population that included blasts of each of the cell lineages, megakaryocytes, plasma

cells, and basophils, mean 1.6%; SD .+-. 1.2; 95% limits, 1.3-1.9%. The percentage of reticulocytes in bone marrow aspirates from 50 normal donors correlated with the reticulocyte frequency in the peripheral blood of these donors. However, the mean frequency of reticulocytes was significantly greater ($p < 0.0001$) in bone marrow (mean 2.19%; SD .+-. 0.88) than in peripheral blood (mean 1.71%; SD .+-. 0.88). The technique could discriminate between immature and mature reticulocytes based on the brighter staining with both Thiazole-Orange and LDS-751 of the immature reticulocytes. This was confirmed by cell sorting of both reticulocyte populations, which revealed larger clumps of New Methylene Blue staining material in the brighter Thiazole-Orange and LDS-751 stained reticulocytes. The immature reticulocytes were present in normal bone marrow, but not in normal peripheral blood. As expected, a significantly greater frequency of nucleated cells was found in bone marrow aspirates (mean 0.85%; SD .+-. 0.59) than in peripheral blood (mean 0.20%; SD .+-. 0.11). The frequency of platelets was significantly lower in bone marrow (mean 1.24%; SD .+-. 0.69) than in peripheral blood (mean 2.94%, SD .+-. 1.14). **Flow cytometric** bone marrow analysis can provide clinical laboratories with a technique that generates quantitative bone marrow cell differentials and potentially can reduce the need for light microscopic examination of bone marrow smears.

TI Bone marrow cell differential counts obtained by multidimensional **flow cytometry**.

AB Five-dimensional **flow cytometric** analysis of normal bone marrow aspirates was utilized to determine the frequency of neutrophils, eosinophils, monocytes, lymphocytes, nucleated erythrocytes, reticulocytes, platelets, and a cell population that included blasts of each of the cell lineages, megakaryocytes, plasma cells, and basophils. Each of these bone marrow cell populations had unique features with respect to forward **light scatter**, orthogonal **light scatter**, and staining with Thiazole-Orange, LDS-751, and CD45 labeled with **Phycoerythrin** (**PE**). The identity of the cell populations was verified by sorting each of the cell populations and subsequent light microscopic examination of the cells. The frequencies of the nucleated bone marrow cell subpopulations of 50 normal donors were for neutrophils, mean 72.3%; SD .+-. 5.1; 95% limits, 70.9-73.8%; eosinophils, mean 1.8%; SD .+-. 1.3; 95% limits, 1.4-2.1%; monocytes, mean, 2.8%; SD .+-. 1.2; 95% limits, 2.5-3.1%; lymphocytes, mean 12.1%; SD .+-. 3.6; 95% limits 11.1-13.2%; nucleated erythrocytes, mean 8.9%; SD .+-. 3.9; 95% limits, 7.8-10.1%; and the cell population that included blasts of each of the cell lineages, megakaryocytes, plasma cells, and basophils, mean 1.6%; SD .+-. 1.2; 95% limits, 1.3-1.9%. The percentage of reticulocytes in bone marrow aspirates from 50 normal donors correlated with the reticulocyte frequency in the peripheral blood of these donors. However, the mean frequency of reticulocytes was significantly greater ($p < 0.0001$) in bone marrow (mean 2.19%; SD .+-. 0.88) than in peripheral blood (mean 1.71%; SD .+-. 0.88). The technique could discriminate between immature and mature reticulocytes based on the brighter staining with both Thiazole-Orange and LDS-751 of the immature reticulocytes. This was confirmed by cell sorting of both reticulocyte populations, which revealed larger clumps of New Methylene Blue staining material in the brighter Thiazole-Orange and LDS-751 stained reticulocytes. The immature reticulocytes were present in normal bone marrow, but not in normal peripheral blood. As expected, a significantly greater frequency of nucleated cells was found in bone marrow aspirates (mean 0.85%; SD .+-. 0.59) than in peripheral blood (mean 0.20%; SD .+-. 0.11). The frequency of platelets was significantly lower in bone marrow

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CT Medical Descriptors:
*bone marrow cell
***flow cytometry**
***leukocyte differential count**
*reticulocyte
adult
article
female
human
human cell
human tissue
male
normal human
priority journal
thrombocyte
Drug Descriptors:
*cd45 antigen: EC, endogenous compound

L141 ANSWER 10 OF 14 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 6
1991:497396 Document No.: BA92:120356. MULTIDIMENSIONAL **FLOW CYTOMETRIC** BLOOD CELL DIFFERENTIAL WITHOUT ERYTHROCYTE LYSIS. TERSTAPPEN L W M M; JOHNSON D; MICKEALS R A; CHEN J; OLDS G; HAWKINS J T; LOKEN M R; LEVIN J. BECTON DICKINSON IMMUNOCYTOMETRY SYSTEMS, 2350 QUME DRIVE, SAN JOSE, CALIF. 95131.. BLOOD CELLS (N Y), (1991) 17 (3), 585-602.
CODEN: BLCEDD. ISSN: 0340-4684. Language: English.

AB Forward **light scattering**, orthogonal **light scattering**, and the fluorescence intensities of unlysed peripheral blood cells, labeled with CD45-**phycoerythrin** and the nucleic acid dyes LDS-751 and thiazole orange, were measured simultaneously, utilizing a **flow cytometer**. Erythrocytes, reticulocytes, platelets, neutrophils, eosinophils, basophils, monocytes, lymphocytes, nucleated erythrocytes, and immature nucleated cells occupied unique positions in the five-dimensional space created by the listmode storage of the five independent parameters. A software program was developed which identified and enumerated each of these cell populations. Platelets in this study were identified by LDS-751 staining, in addition to their forward and orthogonal **light-scattering** characteristics. Validation of this approach was obtained by demonstrating that all CD41- or CD42-expressing platelets also stained with LDS-751. Furthermore, the staining by LDS-751 did not change following platelet activation with ADP. The quantification of erythrocytes, platelets, neutrophils, eosinophils, monocytes, and lymphocytes correlated well with data obtained with a commerical hematology whole blood analyzer (H-1). Reproducibility of the identification of these populations was shown by repeated meaurement of the same sample and by staining and analysis of multiple aliquots of identical blood samples. Stability studies demonstrated that 8 hours after blood collection, the number of damaged cells increased. This could be measured by a greater thiazole orange uptake by the damaged cells. This investigation demonstrates the feasibility of multidimensional **flow cytometric** blood cell differentiation for an automated whole blood cell analysisi without the necessity of erythrocyte lysis. The ability to simultaneously identify

reticulocytes, nucleated erythrocytes, and immature nucleated cells in one measurement is unique and promises to be a powerful tool for the assessment of abnormal blood samples.

TI MULTIDIMENSIONAL **FLOW CYTOMETRIC** BLOOD CELL DIFFERENTIAL WITHOUT ERYTHROCYTE LYSIS.

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IT Miscellaneous Descriptors
HUMAN DIFFERENTIAL **LEUKOCYTE COUNTING** RETICULOCYTE

L141 ANSWER 11 OF 14 MEDLINE DUPLICATE 7

90010175 Document Number: 90010175. A rapid sample preparation technique for **flow cytometric** analysis of immunofluorescence allowing absolute enumeration of cell subpopulations. Terstappen L W; Meiners H; Loken M R. (Becton Dickinson, Monoclonal Center, Mountain View, CA 94039..) JOURNAL OF IMMUNOLOGICAL METHODS, (1989 Sep 29) 123 (1) 103-12. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB A simple and rapid method was developed for immunofluorescence measurements of cells by **flow cytometry** which does not require washing procedures, permitting absolute enumeration of cell subpopulations. Peripheral blood cells were labeled with fluorescein and **phycoerythrin** conjugated monoclonal antibodies and the nucleic acid stain LDS-751. Distilled water was added following incubation to induce erythrocyte lysis by hypotonic shock. After lysis for 30 s the tonicity of the sample was increased followed by measurement on the

flow cytometer. The leukocyte populations were clearly resolved in the correlation of forward and orthogonal **light scattering**. The immunofluorescence resolution of the labeled leukocytes was equivalent to NH4Cl and a commercial lysing preparation. Absolute number of leukocytes and percentage of leukocyte subpopulations determined with this procedure correlated well with the results obtained with a clinical hematology analyzer. Cell recovery and preservation of cellular characteristics of three different procedures for lysing the human erythrocytes were compared. The LDS-751 permitted the discrimination of intact cells from residual erythrocyte ghosts, platelets and damaged nucleated cells. A considerable loss of cells was found for both NH4Cl and commercial lysing solution; the samples prepared by NH4Cl lysing had a selective loss of lymphocyte subpopulations as compared with the other two techniques. In contrast to the two procedures in which multiple washing steps are involved, the no wash, hypotonic lysis procedure provided a means of obtaining absolute numbers of leukocyte subpopulations identified by combining **light scattering** and immunofluorescence characteristics with no centrifugation steps required.

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CT Check Tags: Human
Ammonium Chloride: PD, pharmacology
Antigens, Differentiation, B-Lymphocyte
Antigens, Differentiation, T-Lymphocyte
***Flow Cytometry: MT, methods**
Fluorescent Antibody Technique

***Leukocyte Count**
Light
Receptors, Antigen, T-Cell: AN, analysis
Scattering, Radiation

L141 ANSWER 12 OF 14 MEDLINE DUPLICATE 8
86267192 Document Number: 86267192. Characteristics of monoclonal antibody measurements in human peripheral blood. Thornthwaite J T; Seckinger D; Rosenthal P; Vazquez A. ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1986)
468 144-59. Journal code: 5NM. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB Three areas of monoclonal antibody measurements using **flow cytometry** have been presented. These include a description of a dual immunofluorescent method for measuring two antibodies simultaneously, the effects of blood storage on enumeration of helper (H) and suppressor (S) cells, and the relationship between absolute **lymphocyte count** and H/S ratio in both control and AIDS patients. These studies reveal that a dual immunofluorescent labeling method is useful for enumerating lymphocytes from peripheral blood which bear the helper, suppressor and/or thymus-derived (T) cell receptors. Fluorescein (FL)-conjugated Leu-3a + 3b antibodies were used to enumerate helper T-lymphocytes, while the B-**phycoerythrin** (B-**PE**)-conjugated Leu-2a antibodies were utilized for enumerating suppressor T-lymphocytes. Dual immunofluorescently stained lymphocytes, prepared from whole blood, were analyzed by **flow cytometry**. Two **light scatter** parameters, (forward and 90 degree scatter) were used to define the lysed erythrocyte, lymphocyte, monocyte, and granulocyte populations. Only the lymphocytes were analyzed for dual immunofluorescence activity. The helper and suppressor distributions from 167 control patients were as follows: The average percentage +/- SD of the helper and suppressor cells were 42.8 +/- 7.5 and 21.6 +/- 6.4, respectively. The H/S ratio was 2.17 +/- .75. These studies show that the H/S ratio can be determined in a single preparative sample and analyzed by dual immunofluorescence in a single **flow cytometric** analysis even though the H/S ratio may vary from normal during a disease condition. The dual immunofluorescent assay enables one to correlate the activities of two antibodies against cell surface receptors and allows the measurement of a large number of samples in a minimal time. This study also compared the effects of anticoagulant, storage time, and temperature on the phenotypic determination of the percentages of helper and suppressor T-lymphocytes in human peripheral blood. Blood was drawn in ACD, heparin, and EDTA and stored for up to 4 days at room temperature or 4 degrees C. Phenotypic determination of helper/suppressor lymphocytes was most stable for ACD or heparinized blood at room temperature. Marked changes were observed in the percentages of helper cells at 4 degrees C, whereas the percentages of suppressor cells did not change appreciably regardless of the anticoagulant storage time or temperature. Finally, the relationship between ALC and the H/S ratio in control and AIDS patients was determined. The ALC varied considerably in both control and patient populations as a function of time. Conversely, the H/S ratio remained constant.(ABSTRACT TRUNCATED AT 400 WORDS)

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dual immunofluorescent method for measuring two antibodies simultaneously, the effects of blood storage on enumeration of helper (H) and suppressor (S) cells, and the relationship between absolute **lymphocyte count** and H/S ratio in both control and AIDS patients. These studies reveal that a dual immunofluorescent labeling method is useful for enumerating lymphocytes from peripheral blood which bear the helper, suppressor and/or thymus-derived (T) cell receptors. Fluorescein (FL)-conjugated Leu-3a + 3b antibodies were used to enumerate helper T-lymphocytes, while the B-**phycoerythrin** (B-**PE**)-conjugated Leu-2a antibodies were utilized for enumerating suppressor T-lymphocytes. Dual immunofluorescently stained lymphocytes, prepared from whole blood, were analyzed by **flow cytometry**. Two **light scatter** parameters, (forward and 90 degree scatter) were used to define the lysed erythrocyte, lymphocyte, monocyte, and granulocyte populations. Only the lymphocytes were analyzed for dual immunofluorescence activity. The helper and suppressor distributions from 167 control patients were as follows: The average percentage +/- SD of the helper and suppressor cells were 42.8 +/- 7.5 and 21.6 +/- 6.4, respectively. The H/S ratio was 2.17 +/- .75. These studies show that the H/S ratio can be determined in a single preparative sample and analyzed by dual immunofluorescence in a single **flow cytometric** analysis even though the H/S ratio may vary from normal during a disease condition. The dual immunofluorescent assay enables one to correlate the activities of two antibodies against cell surface receptors and allows the measurement of a large number of samples in a minimal time. This study also compared the effects of anticoagulant, storage time, and temperature on the phenotypic determination of the percentages of helper and suppressor T-lymphocytes in human peripheral blood. Blood was drawn in ACD, heparin, and EDTA and stored for up to 4 days at room temperature or 4 degrees C. Phenotypic determination of helper/suppressor lymphocytes was most stable for ACD or heparinized blood at room temperature. Marked changes were observed in the percentages of helper cells at 4 degrees C, whereas the percentages of suppressor cells did not change appreciably regardless of the anticoagulant storage time or temperature. Finally, the relationship between ALC and the H/S ratio in control and AIDS patients was determined. The ALC varied considerably in both control and patient populations as a function of time. Conversely, the H/S ratio remained constant.(ABSTRACT TRUNCATED AT 400 WORDS)

CT Check Tags: Human; Support, Non-U.S. Gov't
Acquired Immunodeficiency Syndrome: BL, blood
*Antibodies, Monoclonal
Citrates
Edetic Acid
Flow Cytometry
Fluorescent Antibody Technique
Heparin
***Leukocyte Count: MT, methods**
*Lymphocytes: CY, cytology
Scattering, Radiation
Temperature

L141 ANSWER 13 OF 14 MEDLINE
85049966 Document Number: 85049966. The effects of anticoagulant and temperature on the measurements of helper and suppressor cells.

Thornthwaite J T; Rosenthal P K; Vazquez D A; Seckinger D. DIAGNOSTIC IMMUNOLOGY, (1984) 2 (3) 167-74. Journal code: DID. ISSN: 0735-3111. Pub.
country: United States. Language: English.

AB This study compared the effects of anticoagulant, storage time, and temperature on the phenotypic determination of the percentages of helper and suppressor Thymus-derived (T) lymphocytes in human peripheral blood. Blood was drawn in ACD, heparin, and EDTA and stored for up to 4 days at room temperature or 4 degrees C. A dual immunofluorescent labeling method, using fluoresceinated-helper (LEU 3a + b) and B-**phycoerythrinated** -suppressor (LEU 2a) antibodies, was used to simultaneously determine the percentages of the lymphocyte types in whole blood preparations by **flow cytometry. Light scatter** distributions were stable for ACD or heparinized blood at room temperature, whereas EDTA or 4 degrees C caused changes in the granulocyte distributions. Phenotypic determination of helper/suppressor lymphocytes was most stable for ACD or heparinized blood at room temperature. However, heparinized and EDTA blood showed marked decreases in the percentages of helper cells at 4 degrees C, and EDTA blood stored at room temperature showed an increase in helper cells. The percentages of suppressor cells did not change appreciably regardless of the anticoagulant, storage time, or temperature.

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CT Check Tags: Human; Support, Non-U.S. Gov't
*Anticoagulants: PD, pharmacology
Blood Preservation
Drug Stability
Flow Cytometry
Fluorescent Antibody Technique
***Leukocyte Count**
Leukocyte Count: ST, standards
Light
Scattering, Radiation
*T-Lymphocytes, Helper-Inducer
*T-Lymphocytes, Suppressor-Effector
*Temperature

L141 ANSWER 14 OF 14 MEDLINE

84253637 Document Number: 84253637. Dual immunofluorescent analysis of human
peripheral blood lymphocytes. Thornthwaite J T; Seckinger D; Sugarbaker E V; Rosenthal P K; Vazquez D A. AMERICAN JOURNAL OF CLINICAL PATHOLOGY, (1984 Jul) 82 (1) 48-56. Journal code: 3FK. ISSN: 0002-9173. Pub. country: United States. Language: English.

AB These studies reveal that a dual immunofluorescent labeling method is useful for enumerating cells from human peripheral blood that bear the helper, suppressor, and/or T-cell receptors. Fluorescein (FL)-conjugated Leu-3a + 3b antibodies were used to enumerate Helper (H) T-lymphocytes, while the B-**phycoerythrin** (B-**PE**)-conjugated Leu-2a antibodies were utilized for quantitating suppressor (S) T-lymphocytes. FL-conjugated Leu-4 antibodies were used to measure the T-lymphocyte activity. Dual immunofluorescent stained lymphocytes, prepared either from whole blood or by Ficoll-Hypaque, gradient cell separation, were analyzed by **flow cytometry**. Two **light scatter** parameters, forward and 90 degree, were used to define the lysed erythrocyte, lymphocyte, monocyte, and granulocyte populations. Only the lymphocytes were analyzed for dual immunofluorescence activity. The helper, suppressor, and T-lymphocyte distributions from 100 controls were as follows: The average percentages +/- SD of the helper and suppressor cells were 41.2 +/- 7.2 and 23.0 +/- 7.2, respectively. The H/S ratio was 1.99 +/- 0.77, while the T-cell distribution on 28 patients was 71.4 +/- 7.7. The Ficoll-Hypaque purified lymphocytes and lysed whole blood lymphocytes compared favorably in their H/S ratios. A comparison was made between the percentages of helper and suppressor cells enumerated by fluorescent microscopy and **flow cytometry** in which correlation coefficients of 0.80 and 0.86 were determined, respectively. These studies show that helper and suppressor T-lymphocytes can be quantitated simultaneously by **flow cytometry**, which enables one to correlate the phenotypic activities of two antibodies against cell surface receptors and permits the measurement of a large number of samples in a minimal time.

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number of samples in a minimal time.
CT Check Tags: Comparative Study; Human; Male
Adult
*Antibodies, Monoclonal: DU, diagnostic use
Cell Separation
Flow Cytometry
*Fluorescent Antibody Technique
Leukocyte Count
Phenotype
T-Lymphocytes, Helper-Inducer: IM, immunology
*T-Lymphocytes, Helper-Inducer: PA, pathology
T-Lymphocytes, Suppressor-Effector: IM, immunology
*T-Lymphocytes, Suppressor-Effector: PA, pathology

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	55.36	361.14

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